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# INHIBITION OF THE METHYLATION OF HISTAMINE IN CAT BRAIN

By T. WHITE

*From the Institute of Physiology, University of Lund, Sweden*

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It has been known for some years that ring-*N*-methylation constitutes an important pathway for the catabolism of histamine in mammals (Schayer, 1959). Recent studies indicate that brain tissue in particular has a great capacity to methylate histamine. This has been shown for the cat both *in vitro* (Brown, Tomchick & Axelrod, 1959; White, 1959) and *in vivo* (White, 1960). Methylation seems to be the major pathway for histamine catabolism in the brain. There is little information on the inhibition of histamine methylation *in vivo*, although a number of substances have been found to inhibit a purified preparation of the histamine-methylating enzyme *in vitro* (Brown *et al.* 1959; Lindahl, 1960). To the author's knowledge no experiments have been carried out to study the inhibition of the methylation of histamine in the living brain.

The present experiments were performed to see if the formation of methylhistamine from histamine in the brain could be inhibited *in vivo* by two of the substances found to be effective *in vitro*, namely chlorpromazine and cupric ions (Brown *et al.* 1959; Lindahl, 1960). It will be shown that both inhibit the methylation of histamine in the cat's brain.

## METHODS

Cats of either sex weighing 2·5–3·5 kg were anaesthetized with Na pentobarbitone intraperitoneally. Animals pre-treated with chlorpromazine received 30 mg/kg and the others 45 mg/kg. The trachea was cannulated and blood pressure recorded by a mercury manometer connected to a femoral artery.

Perfusion took place from the lateral ventricle to the aqueduct, using a Collison cannula for the cannulation of the lateral ventricle (Bhattacharya & Feldberg, 1958). The position of the aqueductal cannula was confirmed at the end of each experiment by dye injection and by dissection of the brain with the cannula in place. In all experiments the tip of the cannula was in the middle of the aqueduct, or in the caudal half of it. In no experiment was leakage of dye into the fourth ventricle observed. In the experiments where high concentration of cupric chloride (0·01 M) and of chlorpromazine (0·05 M) were present in the fluid infused, the effluent showed slight flocculation or opalescence, which disappeared immediately after the addition of a small amount of 0·1 N-HCl. In the other experiments the effluents were clear and colourless.

The perfusion fluid had the following composition (g/l.): NaCl 9·00, KCl 0·42 and $CaCl_2$ (anhydr.) 0·24. In the following pages this solution will be referred to as Ringer's solution.

The ventricles were first perfused for 30 min with Ringer's solution, or Ringer's solution containing one of the inhibitors, and then with the same solution containing 2 $\mu$g $^{14}$C-histamine base/ml. during 40 min. The infusions were made with a motor-driven syringe at a constant rate of 0·11 ml./min and the total volume of the $^{14}$C-histamine solution infused was 4·5 ml. At the end of the perfusion with $^{14}$C-histamine the ventricles were perfused with 5 ml. of Ringer's solution at a rate of approx. 0·5 ml./min, to wash out radioactive substances. For analysis the combined effluents were used. The whole brain including the cerebellum and the medulla oblongata down to the foramen magnum was excised.

The methods for measuring $^{14}$C-histamine and its radioactive metabolites by isotope dilution technique have been developed by Schayer and others and were similar to those used earlier (White, 1959, 1960). A modification in the treatment of the brain samples was used in the present experiments. The brain was homogenized, after preliminary mincing, in a glass homogenizer with approx. the double volume of 0·1 N-HCl. The homogenate was divided into portions (usually 8 to 10), carrier was added to each, and protein precipitated with trichloroacetic acid. The samples were then filtered and treated as described earlier (White, 1960). Only a brief summary of the procedures will be given here.

The following metabolites of histamine were studied: Methylhistamine (1-methyl-4-($\beta$-aminoethyl)-imidazole), methylimidazoleacetic acid (1-methyl-imidazole-4-acetic acid), and imidazoleacetic acid (imidazole-4(5)-acetic acid).

Histamine was extracted into butanol, converted to pipsyl histamine and counted. Methylhistamine was extracted into chloroform and counted in the form of methylhistamine picrate. Imidazoleacetic acid and methylimidazoleacetic acid were separated from histamine and methylhistamine on a cation exchange column (Dowex-50). Pipsylimidazoleacetic acid and the picrate of methylimidazoleacetic acid were then prepared and counted. All determinations of imidazoleacetic acid were preceded by acid hydrolysis of the samples, and the figures for imidazoleacetic acid therefore denote the total amount, free and conjugated. All samples were recrystallized to constant radioactivity, three to six recrystallizations being needed in most cases. The samples of methylhistamine in the perfusate sometimes had to be recrystallized even more often, because of the huge excess of radioactive histamine.

The measurements of radioactivity were made under infinite-thickness conditions in a gas-flow counter with a background activity of 21–24 counts/min. At least 900 counts were taken of each sample after each recrystallization. Differences in counting rates smaller than those corresponding to 0·2–0·3% in the tables are not considered significant.

$^{14}$C-histamine dihydrochloride labelled in the 2-position of the imidazole ring was purchased from the Radiochemical Centre, Amersham, Bucks. After dilution with the standard amount of inert carrier histamine (66·4 mg of the dihydrochloride) and precipitation as the dipicrate, this $^{14}$C-histamine gave 3200 counts/min/$\mu$g base, counted at infinite thickness in the flow counter.

Chlorpromazine was used in the form of the hydrochloride (AB Leo, Hälsingborg, Sweden). The figures for the doses of chlorpromazine refer to the salt. Copper was used in the form of $CuCl_2 . 2H_2O$, containing 37·3% copper.

## RESULTS

The control experiments (Table 1) showed that collection of the effluent from the aqueduct instead of from the cisterna magna caused slightly less methylated histamine metabolites (methylhistamine and methylimidazoleacetic acid) to be formed than in the experiments reported earlier (White, 1960). The methylation that did occur was still great enough to provide a basis for experiments on the inhibition of histamine methylation.

It has recently been shown by Lindahl (1960) that cupric ions inhibit a purified preparation of a histamine-methylating enzyme *in vitro*. In seven experiments the effect of cupric chloride on the methylation of histamine in the cat's brain was studied (Table 2). The cupric salt was administered intravenously (Expts. 5 and 6) and by ventricular perfusion (Expts. 1–4), and also by both routes simultaneously (Expt. 7). In Expts. 5 and 6 of Table 2 cupric chloride, dissolved in 0·9 % NaCl solution, was infused into a femoral vein at a rate of 0·12 mg copper/min for 60 min before and also during the ventricular perfusion with $^{14}$C-histamine. In these experiments the methylation of histamine (i.e. formation of methylhistamine and methylimidazoleacetic acid) was inhibited by approx. 35 %.

TABLE 1. Catabolism of $^{14}$C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused $^{14}$C-histamine recovered as histamine and metabolites

| Expt. no. | | Histamine | Methyl-histamine | Methyl-imidazole-acetic acid | Imidazole-acetic acid | Sum of methyl derivatives | Total recovery |
|---|---|---|---|---|---|---|---|
| 1 | Effluent | 83 | 2·9 | 0·4 | 0·2 | 9·7 | 94 |
| | Brain | 0·4 | 3·5 | 2·9 | 0·2 | | |
| 2 | Effluent | 89 | 2·2 | 0·3 | 0·1 | 8·5 | 98 |
| | Brain | 0·5 | 3·8 | 2·2 | 0·1 | | |
| 3 | Effluent | 83 | 2·0 | 0·4 | 0·6 | 9·8 | 94 |
| | Brain | 0·3 | 3·5 | 3·9 | 0·2 | | |
| 4 | Effluent | 83 | 2·2 | 0·1 | 0·3 | 9·2 | 94 |
| | Brain | 0·8 | 4·8 | 2·1 | 0·2 | | |
| 5 | Effluent | 87 | 2·7 | 0·4 | 0·2 | 9·1 | 96 |
| | Brain | 0·2 | 3·5 | 2·5 | 0·2 | | |

Greater inhibition was observed when the cupric chloride was added to the perfusion fluid (Expts. 1–4 of Table 2), 0·001 M inhibiting approx. 55 % and 0·01 M approx. 80 %. It can be seen that in the experiments showing the smallest formation of methylhistamine (Expts. 3 and 4 of Table 2) the brain contained more unchanged histamine than in the control experiments (Table 1).

In Expt. 7 of Table 2 cupric chloride was infused both intravenously (0·12 mg copper/min) and intraventricularly. This experiment showed an inhibition of the histamine methylation slightly greater than that observed after each treatment alone.

Comparison between the results with and without copper in the perfusion fluid would only be valid if the concentrations of $^{14}$C-histamine were the same. The formation of a histamine-copper complex in the copper-containing perfusion fluid (Eichler & Meyer, 1948) might diminish the concentration of free histamine. In the experiments where cupric chloride in a concentration of 0·01 M was infused the fluid remaining in the syringe after the termination of the perfusions was assayed for histamine activity on the isolated guinea-pig ileum against a corresponding solution lacking

TABLE 2. The effect of cupric chloride on the catabolism of $^{14}$C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused $^{14}$C-histamine recovered as histamine and metabolites

| Expt. no. | $CuCl_2$ | | Histamine | Methyl-histamine | Methyl-imidazole-acetic acid | Imidazole-acetic acid | Sum of methyl derivatives | Total recovery |
|---|---|---|---|---|---|---|---|---|
| 1 | $10^{-3}$ M intraventricularly | Effluent | 83 | 0·5 | 0·0 | 0·9 | 4·7 | 89 |
| | | Brain | 1·0 | 3·2 | 1·0 | 0·0 | | |
| 2 | $10^{-3}$ M intraventricularly | Effluent | 83 | 0·5 | 0·1 | 0·2 | 3·5 | 87 |
| | | Brain | 0·6 | 1·8 | 1·1 | 0·1 | | |
| 3 | $10^{-3}$ M intraventricularly | Effluent | 91 | 0·3 | 0·0 | 1·5 | 1·9 | 96 |
| | | Brain | 1·8 | 1·5 | 0·1 | 0·0 | | |
| 4 | $10^{-3}$ M intraventricularly | Effluent | 88 | 0·5 | 0·0 | 0·2 | 1·8 | 92 |
| | | Brain | 1·5 | 1·2 | 0·1 | 0·0 | | |
| 5 | Intravenously | Effluent | 94 | 1·3 | 0·2 | 0·4 | 6·0 | 101 |
| | | Brain | 0·6 | 2·9 | 1·6 | 0·2 | | |
| 6 | Intravenously | Effluent | 87 | 1·5 | 0·2 | 0·2 | 5·7 | 93 |
| | | Brain | 0·3 | 2·0 | 2·0 | 0·1 | | |
| 7 | $10^{-3}$ M intraventricularly + intravenously | Effluent | 93 | 0·2 | 0·0 | 0·3 | 3·4 | 97 |
| | | Brain | 0·8 | 2·4 | 0·8 | 0·0 | | |

TABLE 3. The effect of chlorpromazine on the catabolism of $^{14}$C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused $^{14}$C-histamine recovered as histamine and metabolites

| Expt. no. | Chlorpromazine | | Histamine | Methyl-histamine | Methyl imidazole-acetic acid | Imidazole-acetic acid | Sum of methyl derivatives | Total recovery |
|---|---|---|---|---|---|---|---|---|
| 1 | $5 \times 10^{-3}$ M intraventricularly | Effluent | 74 | 0·3 | 0·0 | 0·2 | 7·1 | 84 |
| | | Brain | 2·1 | 4·6 | 2·2 | 0·0 | | |
| 2 | $5 \times 10^{-3}$ M intraventricularly | Effluent | 67 | 0·5 | 0·2 | 0·2 | 15 | 84 |
| | | Brain | 2·0 | 10·4 | 3·5 | 0·0 | | |
| 3 | 3 × 50 mg/kg intramuscularly | Effluent | 82 | 1·8 | 0·2 | 3·1 | 5·7 | 89 |
| | | Brain | 0·8 | 2·7 | 1·0 | 0·1 | | |
| 4 | 3 × 50 mg/kg intramuscularly | Effluent | 92 | 0·8 | 0·0 | 0·3 | 3·8 | 96 |
| | | Brain | 0·3 | 1·5 | 1·5 | 0·0 | | |
| 5 | 3 × 50 mg/kg intramuscularly | Effluent | 84 | 1·2 | 0·1 | 0·3 | 6·6 | 91 |
| | | Brain | 0·4 | 3·3 | 2·0 | 0·0 | | |
| 6 | 3 × 12·5 mg/kg intramuscularly | Effluent | 91 | 1·8 | 0·4 | 0·0 | 6·6 | 98 |
| | | Brain | 0·2 | 2·0 | 2·4 | 0·2 | | |
| 7 | 3 × 12·5 mg/kg intramuscularly | Effluent | 86 | 1·6 | 0·2 | 0·3 | 6·7 | 93 |
| | | Brain | 0·5 | 3·0 | 1·9 | 0·2 | | |

copper, and also against a reference solution prepared from non-radioactive histamine. No difference could be detected between the radioactive solutions with and without copper. The concentration of histamine in the perfusion fluid differed less than 10% from the expected value (2 $\mu$g/ml.). It was concluded that the addition of copper did not influence the amount of free biologically active $^{14}$C-histamine in the perfusion fluid.

When chlorpromazine was perfused through the ventricles (Expts. 1 and 2 of Table 3), some depression of the blood pressure occurred, and it was difficult to obtain a constant flow through the ventricular system. It would seem that ventricular perfusion was not a suitable method for the administration of high concentrations of chlorpromazine to the brain.

In five experiments (3–7 of Table 3) cats were given three intramuscular injections of chlorpromazine, receiving two injections on the day before the ventricular perfusion, and the third about 2 hr before the start of the ventricular perfusion. The rather large doses employed caused changes in the behaviour of the animals. They became drowsy and passive, and the gait was somewhat inco-ordinated, but they were conscious and their reactions seemed otherwise adequate. The values obtained for the catabolism of $^{14}$C-histamine in the brain in these experiments indicated less formation of methylhistamine and methylimidazoleacetic acid than in the control experiments (Table 1).

## DISCUSSION

The methylation of histamine can take place in several regions of the brain, as judged from *in vitro* experiments on minced tissue (White, 1959) and on purified enzyme preparations (Brown *et al.* 1959). Histamine perfused through the cerebral ventricles of anaesthetized cats has been shown to enter the brain tissue surrounding the ventricular cavities, and this uptake of histamine shows differences dependent on the structures of the ventricular wall, the highest concentrations of histamine being found in the grey matter lining the ventricles (Draškoci, Feldberg, Fleischhauer & Haranath, 1960). It may be assumed that other substances also present in the ventricular fluid enter these regions, and that in the present experiments cupric ions travel together with histamine in the brain tissue to reach the sites where the histamine is methylated.

It is evident from a comparison of Table 1 with previous findings (White, 1960) that results obtained by collection of the effluent from the aqueduct are more consistent than those obtained by collection from the cisterna magna. These two techniques differ in that the fourth ventricle and part of the subarachnoid space are perfused in the latter method, but not in the former. It is also evident that the aqueductal perfusions tend to give lower figures for the sum of methylated derivatives. This may be

explained by methylation in the cerebellum and the caudal part of the brain stem (Brown *et al.* 1959), which are not reached by the perfusion fluid when the aqueduct is cannulated. When the effluent is collected from the cisterna magna the cerebellum and the brain stem may be reached, in different experiments, by varying amounts of $^{14}$C-histamine (Bhawe, 1958), and therefore variable amounts of $^{14}$C-methylhistamine may be formed. Further, the drainage through the cisternal cannula of fluid containing the radioactive substances may vary. This could also explain the variable and low values for the total recovery in the cisternal perfusions.

The present experiments *in vivo* demonstrate that cupric chloride and chlorpromazine decrease the amount of methylhistamine and methylimidazoleacetic acid formed during the experiment. The experiments do not supply precise evidence as to the mechanism responsible for this effect. It should be noted that the doses employed were rather large, and that the drugs may influence various functions in the organism. The greatest inhibition of the methylation of histamine was observed when cupric chloride was perfused through the ventricles. In this case the possibility must be considered that the cupric salt so altered the qualities of the lining surfaces of the ventricles that less histamine penetrated into the brain tissue, thereby causing less methylated histamine derivatives to be formed. If this had been so, and the methylating capacity of the tissue was still intact one would expect to find less unchanged histamine in the tissue. On the other hand, if the penetration of histamine into the tissue from the ventricles had been normal, but the methylating process inhibited, then one would expect to find more unchanged histamine in the tissue, and less methylated derivatives. It can be seen that in Expts. 3 and 4 of Table 2 the findings agree best with the latter alternative. Thus it seems likely that the limiting factor in the experiments with intraventricular copper is the methylating capacity of the brain tissue rather than the penetration of histamine into the tissue.

Cats pre-treated with intramuscular injections of chlorpromazine showed less methylation of histamine during aqueductal perfusions than the control animals. Chlorpromazine is present in the rat brain several hours after an intramuscular injection of the drug (Wechsler & Forrest, 1959). The amount found in the brain after a dose slightly larger than that employed in the present report corresponds to a concentration of chlorpromazine greater than that producing inhibition of histamine methylation *in vitro* (Brown *et al.* 1959). The complexity of action of chlorpromazine in the intact organism makes the interpretation of any observed effect of the drug difficult, but the available evidence would agree with the assumption that chlorpromazine inhibits histamine methylation in the brain by inhibition of the histamine-methylating enzyme.

SUMMARY

1. Cupric chloride inhibited the methylation of histamine in the brain after intravenous and intraventricular administration.

2. Chlorpromazine injected intramuscularly inhibited the methylation of histamine in the brain.

3. Possible mechanisms of these actions of copper and chlorpromazine are discussed.

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# SOME EFFECTS OF HISTAMINE AND TWO HISTAMINE METABOLITES ON THE CAT'S BRAIN

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Brain tissue of several mammalian species including the cat can metabolize histamine by means of methylation of histamine on the imidazole ring (Brown, Tomchick & Axelrod, 1959; White, 1959, 1960). The resulting product, methylhistamine (1-methyl-4-($\beta$-aminoethyl)-imidazole), is known to have little or no biological activity in comparison with histamine. This has been demonstrated on various test substrates sensitive to histamine, such as the isolated guinea-pig ileum, the cat's blood pressure, and the bronchial resistance of the guinea-pig (Lee & Jones, 1949; Westling, 1957). Therefore, methylation of histamine is generally considered to be an inactivation of histamine. However, so far no experiments have been reported to show that histamine has effects on the brain that methylhistamine has not.

The present experiments made use of two previous findings on the effect of histamine on brain function, namely the capacity of histamine to change the behaviour of conscious cats after injection into the lateral ventricle (Feldberg & Sherwood, 1954), and the observation by Trendelenburg (1957) that intraventricular histamine produces a blood pressure rise in anaesthetized cats. It will be shown that methylhistamine does, in fact, lack the pharmacological actions of histamine on the brain, as judged by the effects after intraventricular administration.

## METHODS

Three cats of either sex weighing 3·0–3·6 kg were used for the intraventricular injections on unanaesthetized animals. A Collison cannula was implanted into a lateral ventricle as described by Feldberg & Sherwood (1953). Each injection consisted of the drug to be tested dissolved in 0·2 ml. Tyrode solution, washed in with another 0·2 ml. Tyrode solution. Control injections of Tyrode solution had little or no effect on the animals. They either behaved just as they did before the injections, or they were somewhat less active during a few minutes, sometimes exhibiting one or two licking movements. Histamine was used in a dose of 100 $\mu$g and had the following effects: increased respiratory rate, panting, swallowing, retching, vomiting, and defaecation. These signs developed within approx. 5 min and disappeared gradually during the following 30–60 min. During the following hour or so the cat lay down in its cage, and then returned to its normal behaviour. These and other observations were

similar to those reported by Feldberg & Sherwood (1954). Vomiting and defaecation appeared in most experiments but were not constant signs. The increase in respiratory rate was a regular sign that could be quantitatively measured. The respiratory rate was counted at 5 min intervals after each injection for at least 30 min, or until it returned to the starting level. The increase or decrease in respiratory rate was calculated as the difference between the highest or lowest observed value after the injection and the mean of two observations 10 and 5 min before injection.

Injections were made at intervals of 7 days or longer. Each injection of methylhistamine (or methylimidazoleacetic acid) was always preceded and followed by histamine injections.

Nineteen cats of either sex weighing 2·0–3·5 kg were anaesthetized with ether and chloralose (70–80 mg/kg) and used for the ventricular perfusion experiments. Blood pressure was recorded in a femoral artery. The ventricular system was perfused with Tyrode solution from the lateral ventricle to the aqueduct, as described by Bhattacharya & Feldberg (1958). The infusions were made with a motor-driven syringe at a constant rate of 0·24 ml./min. The infusion could be switched over from plain Tyrode solution to Tyrode solution containing histamine, methylhistamine or methylimidazoleacetic acid. These substances were usually perfused for 6 min, and then the perfusion with plain Tyrode solution was resumed. The effluents collected from the aqueduct were clear and colourless. The volume of the effluent was always slightly greater than the volume infused. Perfusion of Tyrode solution alone had little or no effect on the blood pressure.

Five cats anaesthetized as above were used for the ventricular injection experiments. The lateral ventricle was cannulated (Feldberg & Sherwood, 1953) and injections were made as described above for the conscious animals. Blood pressure was recorded in a femoral artery.

The Tyrode solution had the following composition (g/l.): NaCl 8·00, KCl 0·20, $CaCl_2.6H_2O$ 0·40, $MgCl_2.6H_2O$ 0·20, $NaHCO_3$ 1·00, and $NaH_2PO_4$ 0·06. Histamine and methylhistamine (1-methyl-4-(β-aminoethyl)-imidazole) were used as the dihydrochlorides. Methylimidazoleacetic acid (1-methylimidazole-4-acetic acid) was used as the hydrochloride. The doses given refer to the bases.

## RESULTS

### *Intraventricular injections in conscious cats*

Twenty-two injections were given to three cats. Three injections of methylimidazoleacetic acid (100 μg twice, and 400 μg once) and six injections of methylhistamine (100 and 400 μg three times each) had no observable effect on the behaviour of the animals, or caused changes similar to those produced by Tyrode solution alone. Histamine (thirteen injections) increased the respiratory rate by 49–152 respirations/min (corresponding to 100–790%). The effects of methylhistamine and methylimidazoleacetic acid on the respiration were small and variable, the extremes being a decrease of 12 and an increase of 28 respirations/min (corresponding to −40 and +80%).

### *Intraventricular injections in anaesthetized cats*

In three of these experiments it was confirmed that histamine injected intraventricularly raised the arterial blood pressure (Trendelenburg, 1957). The amounts injected were 50, 100 and 100 μg, and the corresponding pressor responses were 15, 40, and 70 mm Hg. In two experiments methylhistamine (100 μg) had no effect on the blood pressure.

*Ventricular perfusions in anaesthetized cats*

When the infusion was switched over to a solution containing histamine (100 $\mu$g/ml.) a rise in blood pressure was regularly observed, similar to that obtained after intraventricular injection (Fig. 1). As a rule the blood pressure began to decline towards the normal level while histamine was still being infused. When histamine was perfused a second time after an interval of 20–50 min the pressor response was much smaller or absent.

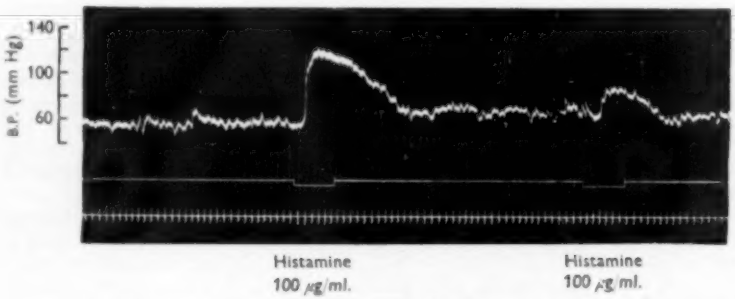


Fig. 1. The effect of ventricular perfusion with histamine on the blood pressure. Cat 2·3 kg, chloralose anaesthesia. Records from above: arterial blood pressure, signal, time marker (minutes). The signal indicates the periods when the perfusion fluid contained histamine.

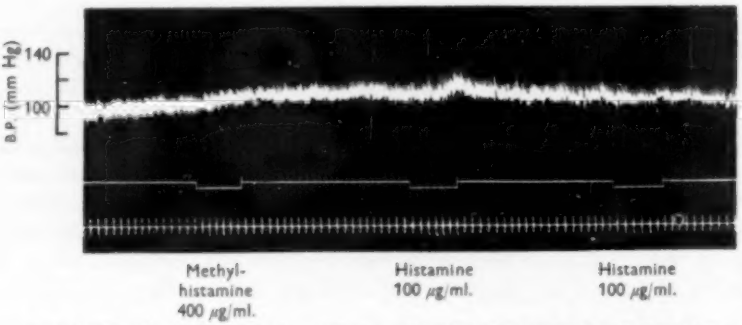


Fig. 2. The effect of ventricular perfusion with histamine and methylhistamine on the blood pressure. Cat 3·4 kg, chloralose anaesthesia. For explanation, see Fig. 1.

Methylhistamine had no significant pressor effect, although a rise of about 10 mm Hg occurred once when methylhistamine was perfused in a concentration of 400 $\mu$g/ml. No depressor responses to either of the substances were observed during ventricular perfusions. When histamine (100 $\mu$g/ml.) was perfused after a perfusion of methylhistamine (400 $\mu$g/ml.)

the pressor response to histamine was negligible (Fig. 2), and the impression was gained that a prior perfusion of methylhistamine (400 $\mu$g/ml.) diminished the response to histamine. This effect of methylhistamine, which was not so obvious after perfusion in a concentration of 100 $\mu$g/ml., was observed in five experiments. However, it does not seem justifiable to state that methylhistamine significantly inhibited the pressor response to histamine, because of the limited number of experiments, and because comparisons could not be made between the effects of repeated perfusion of histamine in the same animal (see above).

In three experiments the effects of methylimidazoleacetic acid (100 $\mu$g/ml. once, and 400 $\mu$g/ml. twice) were examined. Ventricular perfusion with this substance caused no change in the blood pressure. Histamine perfusion (100 $\mu$g/ml.), following perfusion of methylimidazoleacetic acid in a concentration of 400 $\mu$g/ml., gave in one experiment no pressor response, and in the other a small pressor response only (15 mm Hg).

## DISCUSSION

The present experiments show that methylhistamine and methylimidazoleacetic acid are practically inactive in comparison with histamine after intraventricular administration, as judged from the behaviour of conscious cats, and from the blood pressure in anaesthetized cats. The experiments do not supply information on the mode of action of histamine on the brain. The observation that the first response to intraventricular histamine in anaesthetized cats is always greater than the following ones is interesting, in view of the findings by Gertner & Kohn (1959) that the stimulating action of histamine on the superior cervical ganglion exhibits tachyphylaxis. This might be taken to suggest that similar mechanisms are operating in both cases. It was suggested by Trendelenburg (1957) that histamine exerts a direct action on specific ganglion cells of the central nervous system. The present experiments indicate that contact with medullary centres is not essential for the pressor response to intraventricular histamine.

Histamine is present in the brain (Kwiatkowski, 1943; Harris, Jacobsohn & Kahlson, 1952; Clouet, Gaitonde & Richter, 1957), it has pharmacological actions on the brain, and it can be formed in the brain (Holtz & Westermann, 1956; White, 1959, 1960). These facts would seem to lend some support to the hypothesis that histamine has a physiological function in the brain. If this hypothesis is correct, and if the pharmacological effects of intraventricular histamine are related to the physiological actions of histamine, the methylation of histamine in the brain can be regarded as a process of physiological significance.

SUMMARY

1. Histamine, methylhistamine, and methylimidazoleacetic acid were administered into the cerebral ventricles of cats.

2. Only histamine caused changes in the behaviour of conscious cats. In anaesthetized cats ventricular perfusion with histamine gave a blood pressor response, whereas methylhistamine and methylimidazoleacetic acid were inactive.

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# THE REPRESENTATION OF THE VISUAL FIELD ON THE CEREBRAL CORTEX IN MONKEYS

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On the basis of his extensive and elegant anatomical investigations on the visual cortex, Poliak (1932) suggested that a mathematical projection of the retina on the cerebral cortex must exist. Talbot & Marshall (1941) used physiological methods to map the central part of the visual field on to the posterolateral surface of the cortex in the monkey. They devised an index of cortical representation expressed as the increment of the angle, measured radially from the centre of gaze, which is represented on each millimetre of cortex. We have confirmed their observations and have extended the mapping to the buried visual cortex in the horizontal and vertical calcarine fissures. We have preferred to use the reciprocal of their index and to call it the cortical magnification, $M$. When this is measured along radii and at right angles to them, it provides the empirical quantitative relation which Polyak wanted. It also defines the shape and size of the visual receptive field. We have made such a surface, folded it and compared it with the calcarine cortex of the monkey. A preliminary account of this work has been published (Daniel & Whitteridge, 1959).

## METHODS

Out of twenty-two monkeys and baboons, we have had satisfactory records from the posterolateral surface of the cortex in fourteen, from the cortex of the calcarine fissure in eight. The first satisfactory experiments were carried out in 1952, but subsequently there were a number of unsuccessful experiments due to haemorrhage and to prolonged lowering of the blood pressure by pentobarbitone. The last eight experiments on three baboons, three large macaques, a cynomolgus and a vervet monkey have all been successful. We have found operation on small macaques very difficult, as unavoidable blood loss on opening the skull is liable to cause serious lowering of the blood pressure. We have been careful to induce anaesthesia slowly, using Dr C. G. Phillips's method of putting the animal in a small cage in a glass-fronted tank and pumping in nitrous oxide. The moment the animal appears to be unconscious, we have taken it out, given Nembutal (sodium pentobarbitone; Abbott Laboratories) 40 mg/kg, or hexobarbitone intraperitoneally, or chloralose 50–60 mg/kg intravenously. The animal is then put back into the small cage in room air and, after recovering from the nitrous oxide, it quietly becomes drowsy without further handling. In

one experiment in which the bone over the visual cortex was removed at a preliminary operation with aseptic precautions, the monkey readily entered the small cage 2 days later.

Chloralose was used in the last four experiments. No differences in the extent of the responsive area with barbituates and with chloralose have been observed, but the cortex has been in better condition and the blood pressure consistently higher with chloralose. Blood pressure was checked at intervals by means of a cuff on the tail or thigh, with a microphone to detect pulsation. A saline drip was given and, if necessary, 'Dextran' was given intravenously to maintain circulating fluid volume.

The bone over the posterior half of the left hemisphere was removed, giving an exposure from the posterior pole to the central sulcus, and from the mid line as far laterally as possible. The dura was opened under liquid paraffin.

The eye was fixed by four sutures through the limbus, which attached it to a brass ring which enabled it to be moved about. The head was fixed by ear clamps and a jaw piece to a stereotaxic machine, and the Clarke-Horsley plane was levelled with a spirit level in the usual way. In experiments since 1958, in addition to a general anaesthetic the monkey was given tubocurarine 0·2 mg/kg I.V. with artificial respiration, as no other way of preventing eye movements was found to be completely effective. Each animal was kept under full anaesthesia for up to 18 hr, when it was killed by an overdose of pentobarbitone and the brain perfused with 10% formol in NaCl solution 0·9 g/100 ml. The brain was removed on the following day, fixed for 3 weeks in 10% formol, embedded in low-viscosity nitrocellulose and cut serially at 100 $\mu$. Sections were stained with Weil's method for myelin and photographed at $\times 7{\cdot}5$ or $\times 10$.

The stereotaxic machine was arranged so that the right eye was at the centre of an Aimark perimeter whose arm had a diameter of 33 cm. The eye was roughly centred by determining the optic axis by the reflexion of an image from the centre of the cornea. This does not, however, determine the visual axis, which is usually about 5° to the nasal side. In the later experiments one electrode was placed on the most lateral and anterior part of the superficial visual cortex and left there throughout the experiment. The eye was then moved by its ring until a light at the centre of the perimeter gave the largest and earliest response obtainable from this electrode. The animal was then given tubocurarine (in the later experiments). Before every observation with an exploring electrode the response from a central light was checked with the fixed electrode. The results of Talbot & Marshall (1941) and our own agree that the fovea is represented at the anterolateral corner of the visual area. As at this point 1° of the visual field is represented over nearly 6 mm of cortex, a minor displacement of the fixed electrode is of little importance during experiments with the peripheral field. The cornea was kept moist, but no contact lens was used.

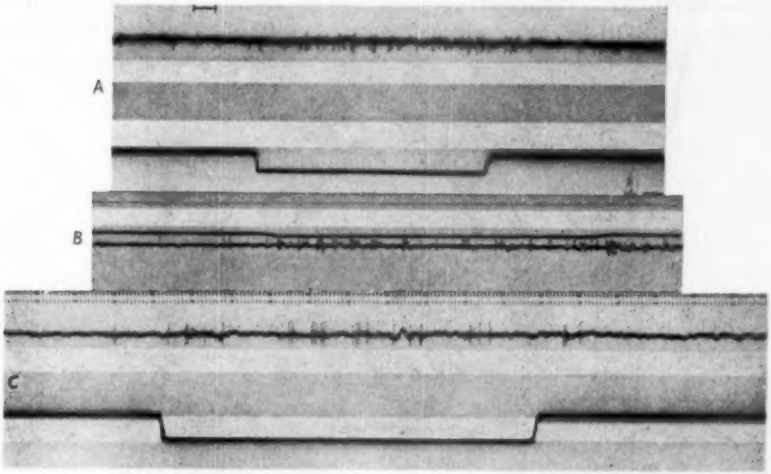
As electrodes we have used steel needles with shafts of 500 $\mu$ diameter with a long taper to less than 10 $\mu$. Such needles leave easily recognized tracks. In this work it was essential to plot as many responses as possible in each experiment, and therefore no systematic attempt was made to record from single units. Instead, we have mapped the centre of the area on the perimeter which gave, from the recording electrode, the largest response of shortest latency. When responses from the same cortical point were repeatedly mapped in the dark, they agreed within 1°. Responses from the same cortical point recorded more than half-an-hour apart agreed to within 1°.

The perimeter gave white round illuminated patches of 38 cd/m$^2$. The area illuminated was approximately a circle whose diameter could be varied from 1 to 10 mm. A transistor photocell excited from the light beyond the shutter provided synchronization of the cathode-ray tube sweep with 'off' and 'on' illumination, but for searching the field a neon tube 5 mm in diameter was used, which flashed for about 5 msec every 2 sec and was triggered from the sweep. This provided too much light for exact localization, and final mapping was done with a light spot of 1 mm diameter which subtended 10′ at the eye, illuminated by 0·38–3·8 cd/m$^2$.

## RESULTS

### *Gross anatomy*

There seems to be very little difference between the calcarine areas in the brains of *Macacus rhesus*, *M. cynomolgus* and the vervet monkey. The baboon has a larger brain, and the calcarine cortex is further from the surface than it is in the macaque, but the general arrangement of the calcarine cortex seems to be the same. Though no electrical recording has




Text-fig. 1

*A*. Increase in a resting discharge in a cortical unit during 'on'. Time marker, 100 msec.

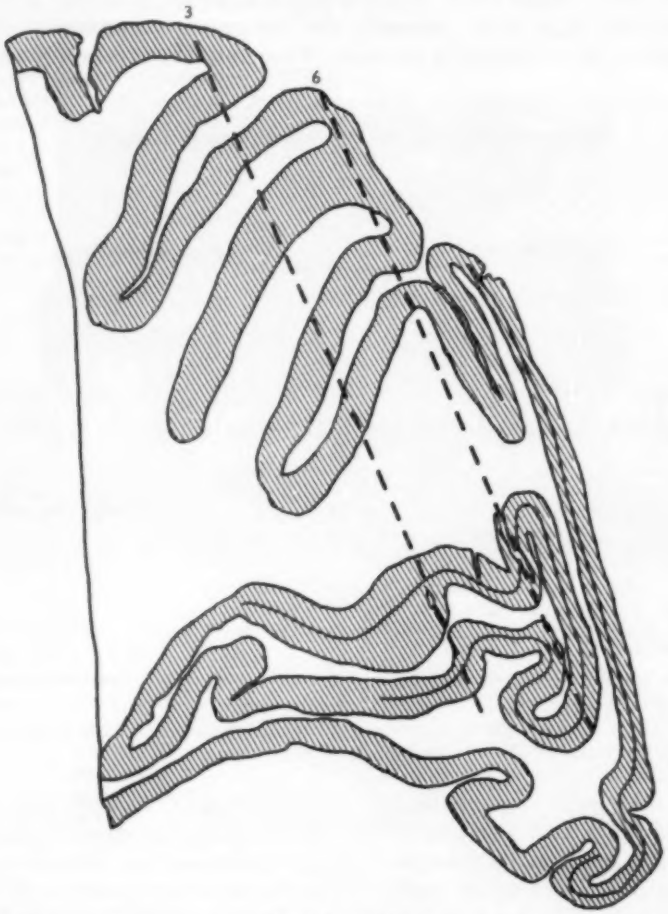
*B*. A number of units from the cortex of a rhesus monkey, 9·7·53. 'On'-effects have a latency of 30 msec.

*C*. 'On'- and 'off'-effects, and an irregular 'on'-discharge in a cortical single unit. Time marker, 1/100 and 1/10 sec. Signal falls at 'on'.

been done on the squirrel monkey and the pigtailed monkey, sections of visual cortex have been examined and show much the same arrangement as in the macaque. On the other hand, the spider monkey, *Ateles*, has much less of the macular cortex visible on the postero-lateral surface, and whereas the vertical limbs of the calcarine fissure lie in the same straight line in the macaque, they form an acute angle with each other in the spider monkey. On these points Polyak (1957) is very informative.

We have little to add to the description by Talbot & Marshall (1941) of the superficial visual cortex. The anterior boundary of the optically

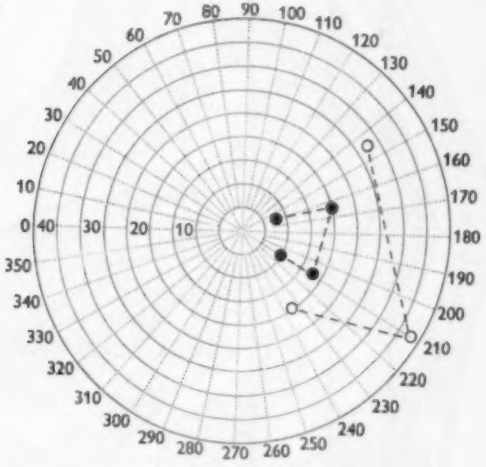
excitable cortex runs 1–2 mm posterior to the large vein that marks the lunate sulcus. A shallow sulcus is constantly seen in sections in the lower part of this area, but it is sometimes difficult to remove enough bone to expose this sulcus at operation. On the medial surface of the brain the calcarine fissure has a ⊣ shape, with a horizontal and two vertical limbs.




Text-fig. 2*A*

Parasagittal section of the occipital region of a baboon's brain. Cortex identifiable as area 17 is marked by a black line. In the stem of the 'mushrooom' there are four needle tracks made by needles slightly oblique to the plane of the section. Two of the tracks, 3 and 6 of the series, have been shown as though projected on to the plane of the section (cf. Text-fig. 2*B*).

This surface has not been explored under direct vision, but histologically area 17 surrounds the vertical limbs of the fissure and forms a narrow band on each side of the more posterior part of the horizontal limb. In sagittal section the calcarine cortex has the shape of a mushroom, with a 'head' and a 'stem'. In sections further from the mid line the 'head' gets smaller, and the most lateral sections show a 'stem' only, frequently cut obliquely (Pl. 1; Text-figs. 2, 3 and 7). The 'head' is frequently asymmetrical about the 'stem' of the calcarine cortex, with the lower part of the 'head' smaller than the upper part. Consistently with this, extra folds of the cortex on the lower wall of the 'stem' of the calcarine fissure are not uncommon. It therefore happens sometimes that area 17 is only found in the upper wall of the 'stem' of the calcarine fissure in the more medial sections.



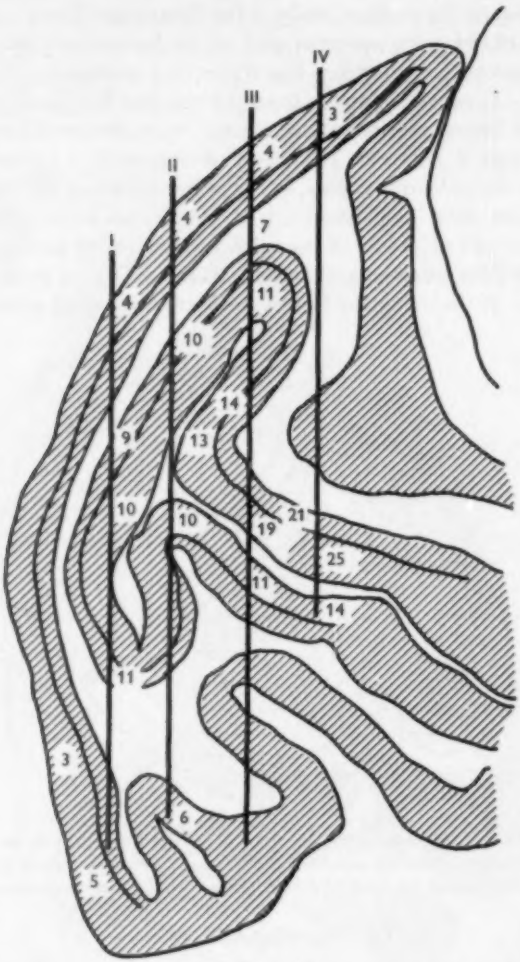

Text-fig. 2*B*

The responses in the visual field from track 3, ○; and from track 6, ●. Owing to the S bend of the 'stem' of calcarine cortex, track 3 gives responses of three points. Track 6 which crosses the head of the 'mushroom' gives responses from four points.

### *The physiological responses*

We have recorded responses from the exposed cortex with a silver ball electrode 2 mm in diameter; from the deeper layers with stainless-steel needles of 5–10 $\mu$ tip, and in later experiments with needles whose tips, produced electrolytically, were not more than 1–2 $\mu$.

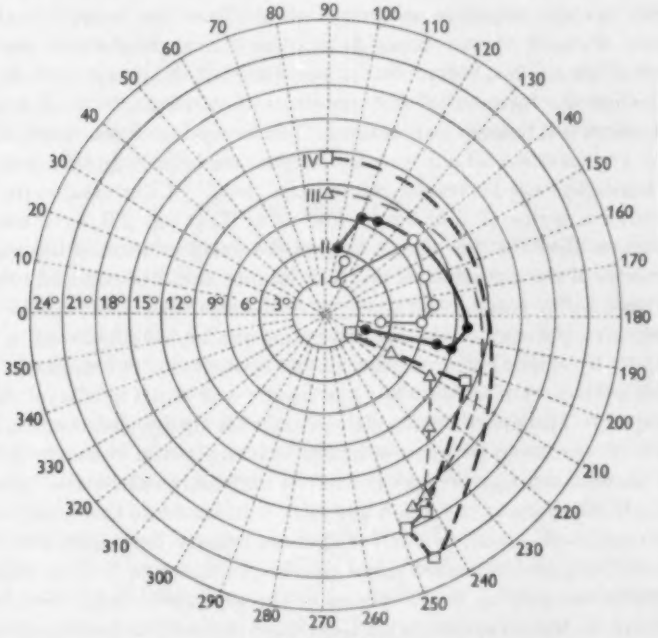
The silver ball regularly gave a response from the cortex, consisting of an earlier and a later wave, with latencies of 30–60 msec and about 100 msec. When the cortex was in good condition, the first wave could be localized to less than 1° on the perimeter, but if the amplitude of the response fell,




Text-fig. 3*A*

A parasagittal section of the occipital region of a baboon's brain, with four needle tracks projected on to it. The figures beside the tracks refer to the radial distance from the fixation point in degrees. Note that the upper lip of the calcarine fissure is concerned with points considerably further from the fixation point than is the lower lip. There is one minor inconsistency; 14° on track 3 should be somewhat lower to correspond with adjacent points (cf. Text-fig. 3*B*).

the point of maximum amplitude of the first wave was not distinguishable over about 3°. With the very fine needles the activity of cells and fibres could readily be recorded and occasionally large single units were obtained. No method has been devised which would permit of immobilization of the cortex over the 4–6 cm² needed for mapping, and it has not yet been possible to map systematically using single units. Further, the amount of detail provided by a fine electrode makes mapping so slow that it is un-




Text-fig. 3*B*

The corresponding points in the visual field. All four needle tracks give at first responses between 5 and 3° out. The roman figures are placed against their last responses, in each case in the upper visual field.

likely that enough points could be dealt with in one experiment. We have found that needles of 5–10 $\mu$ tip give large waves whose components can be easily identified and usually allow one to distinguish white and grey matter.

With a steel electrode in the surface layers of the calcarine cortex a visual response was recorded at about 0·2 mm below the surface. This could be obtained from an area on the perimeter of about 10° in diameter with the neon lamp. Using white light and reducing the area illuminated

to 10′ and the intensity by one or two log. units, we could obtain a response from an area of about 2–4° diameter. These were multiple-unit responses. When the needle was in the calcarine fissure and responses were being obtained from the light source more than 20° out on the perimeter, the receptive field from which responses could be obtained could not be reduced below a diameter of 10°, and the centre of this area could be identified to 1–2°.

Usually when the recording electrode was lowered vertically across the calcarine fissure, responses at first obtained from the lower visual field suddenly changed to the upper field when the electrode had moved a distance of about 200–300 $\mu$. Such 'reversals' of the receptive field were found, when the histological sections became available, to have occurred as the calcarine fissure was crossed. The receptive fields were seldom exactly symmetrical about the horizontal meridian. In the last four experiments the needle tracks crossed the 'head' of the mushroom. This gave either a series of four responses, as in Text-fig. 2*B*, or a series of points as in Text-fig. 3*B*, depending on the exact relation of the track to the surfaces of the calcarine cortex. The latency could be reduced to about 30–35 msec by increasing the strength of the stimulus (Text-fig. 1).

Changes in polarity of the first response can be obtained with a small light spot, by shifting the position of the spot over a few degrees. These changes were most marked when the needle was crossing a layer of area 17 obliquely. The distribution of potentials set up by illuminating small visual fields seems to be more complex than when the visual field is diffusely illuminated and area 17 is excited uniformly. On a few occasions single-cell responses were seen. They have conformed to the usual pattern of 'on' and 'off', 'on', or 'off' responses. Early responses have been elicitable from circumscribed areas on the perimeter of 1–2° in diameter near the fovea, and up to 10° across in the peripheral field. No obvious differences in the receptive fields have been seen when leading from the different layers of the cortex, though very obvious differences were detected in the deeper layers of the colliculus in rabbits (Hamdi & Whitteridge, 1953) and the optic tectum of the pigeon (Hamdi & Whitteridge, 1954).

In addition to these early responses, with a latency of 30–60 msec, late responses or a train of responses were frequently seen. These were always obtainable from a much wider area on the perimeter, even at times from the ipsilateral visual field. They have not been systematically studied (Text-fig. 1).

The further the needle tracks were placed laterally, the nearer the receptive fields were to the horizontal meridian. A number of needle tracks were found to run close to the boundary between areas 17 and 18, a boundary which is quite unmistakable in sections. No visual responses

were obtainable from areas 18 or 19, which were frequently traversed by needle tracks which entered just in front of or behind the lunate sulcus.

When the needle tracks lay in area 17 near to the boundaries between areas 17 and 18, the receptive field was always close to the mid line, i.e. in sectors 90–105° and 255–270° (Text-fig. 3).

### *The ipsilateral representation*

In one baboon there was a cataract in the contralateral eye, and the ipsilateral eye was used instead. As the bridge of the nose is very flat, the nasal field of the ipsilateral eye extends almost as far as does the temporal field. No difference in the magnification factor was found in the ipsilateral eye from the pooled results from all eyes. So far both eyes have not been studied simultaneously beyond the foveal region, nor has the region of the blind spot been specially examined.

### *The magnification factor*

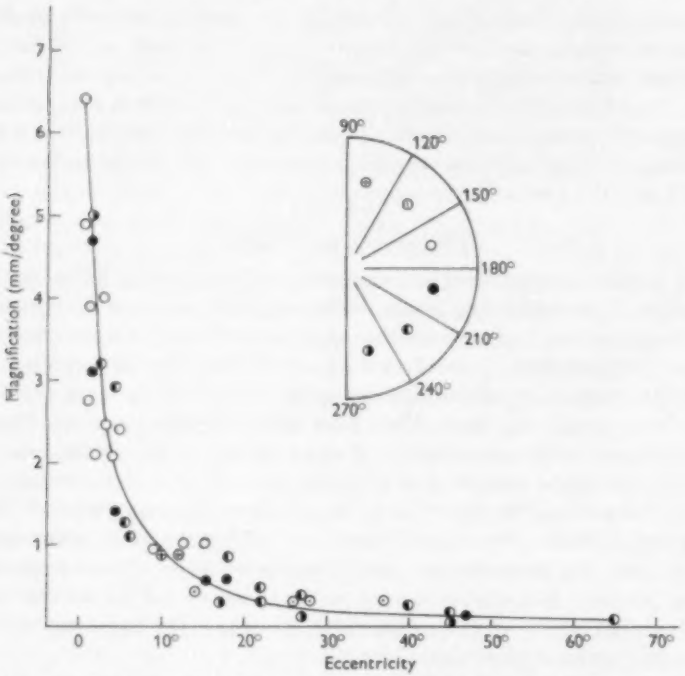
The angular distance apart of two receptive fields and the linear distance between the corresponding points on the calcarine cortex were measured. The magnification factor was obtained as millimetres of cortex concerned with 1° of visual field, by dividing the linear distance by the corresponding angle. The data from all experiments are given in Text-fig. 4, in which the data for six segments, each of 30°, have been plotted separately. There is no significant difference between the six groups of points and one can therefore conclude that there is no significant difference between magnification factors for different radii in the monkey. The magnification factor measured radially over a small distance was found not to differ significantly from the magnification factor measured along the corresponding circumference. No simple equation has been found to fit the data. Neither plotting the data on a semi-log. basis nor plotting reciprocals of the data gives a straight line (Text-fig. 8).

If in fact the magnification factor is the same at all points on a semicircle of latitude in the visual field, and if it has this same constant value along all radii, where they cut this semicircle, the magnification curve should define the area of the visual cortex. The contralateral visual field can be taken as a quarter sphere. This is sufficiently exact for these purposes, as the receptive field continues beyond the points at which it is cut off by eyebrows, etc., in the position of rest of the eyes, and although the visual field extends to 90° out (Chow, Blum & Blum, 1950), the area of cortex devoted to the extreme visual field is probably so small as to be negligible.

One version of the theoretical visual cortex can be constructed from the empirical data by multiplying the length of each semicircle of latitude by the appropriate magnification factor, and then taking any one meridian—

say the horizontal meridian—and multiplying the angular distances between neighbouring semicircles of latitude along it by the appropriate mean value of $M$.

If we give, say, unit radius to the quarter sphere representing the visual field, then an increment of 1° in polar angle (measured from the fixation point) will correspond to an arc on the quarter sphere of length $\pi/180$, and




Text-fig. 4. The magnification factor plotted against eccentricity. The data have been grouped in sectors each of 30°.

will be mapped on to our theoretical visual cortex as an arc of length $M$ mm. The semicircle of polar angle $\theta$ will be represented on the quarter sphere by a curve of length $\pi \sin \theta$, and as the magnification acts uniformly along the length of this curve, its image in the theoretical visual cortex will be a curve of length

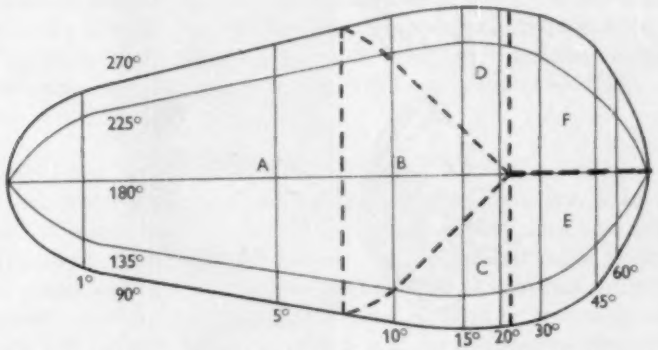
$$M \frac{\pi \sin \theta}{\pi/180} = 180M \sin \theta \text{ mm}.$$

If $\theta_1$ and $\theta_2$ are the polar angles (in degrees) identifying two consecutive semicircles of latitude cutting the basic meridian at $P_1$ and $P_2$, then in the

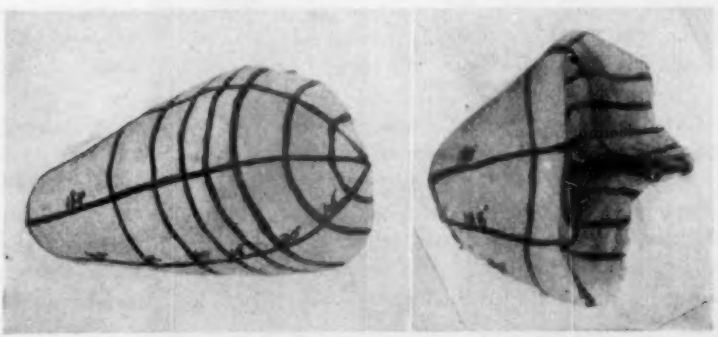
theoretical visual cortex the images of these points will be separated by a distance

$$\frac{M_1+M_2}{2}(\theta_1-\theta_2) \text{ mm.}$$

measured along the image of the same meridian. (Here $M_1$ and $M_2$ are the magnification factors measured at the polar angles $\theta_1$ and $\theta_2$ respectively).

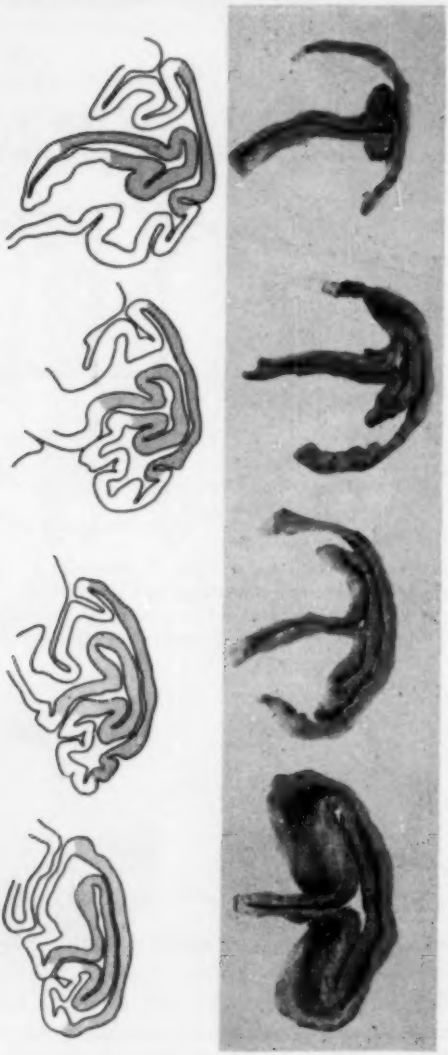



Text-fig. 5. A projection on to a plane of the reconstructed surface for the left hemisphere. Figures refer to the conventional perimeter chart. This surface is folded along the heavy dotted lines so that F touches E, that D and C touch B, and A folds round so that it touches and overlaps the deep surface of B.



Text-fig. 6. Photographs of the model before and after folding. The main horizontal and vertical folds have been completed, but the remainder are only indicated.

This provides the dimensions of a model which can be made in wire covered with paper or cloth and used as a mould for a cast in latex or in 'plasticine'. The cast can then be folded to correspond with the visual cortex (Text-fig. 6). Folding (but not stretching or tearing) will not



*A* *B*

Text-fig. 7

*A*. Parasagittal sections, 2 mm apart, of the occipita region of a baboon's brain. Most lateral section above. Most medial section below.

*B*. Parasagittal sections of a 'plasticine' model made and folded as in Text-fig. 5.

affect its validity. It has been found empirically that if two principal folds are made, one horizontal at 25–90° and one vertical at about 8–9°, with the appropriate connecting folds of Text-fig. 5, the resulting folded surface resembles the visual cortex closely. If serial sections are cut of both, the correspondence of the model with the visual cortex can be seen in Text-fig. 7.

The area of the model can be measured by using strips bounded by semicircles of latitude, and has a value of 1320 $mm^2$. Various measurements of the area of the visual cortex are given in the literature. That given by Clark (1941) for the macaque is 1445 $mm.^2$ Another estimate is 650 $mm^2$, uncorrected for 70 % linear shrinkage (Chow *et al.* 1950).

## DISCUSSION

The technical difficulties in producing a self-consistent plot of the visual field on the calcarine cortex have been almost entirely due to unwanted eye movement. Adequate curarization of an animal kept under full general anaesthesia has been by far the best way of avoiding small movements, and constant checking of the visual fixation by means of a fixed electrode left on the cortex at the foveal representation provides an adequate safeguard. No doubt more accurate mapping could have been attained by leading only from single units in the cortex. It is, however, essential to lead from a very large number of points in each experiment to obtain a useful map, and the time available while the animal remained in good condition made multiunit recording inevitable. This results in leading from larger areas without sharply defined edges, but as the centre of the receptive area can be defined to 1° or less, it causes no serious difficulty.

The density of the degeneration produced in the lateral geniculate body both by small retinal lesions (Clark & Penman, 1934) and by small cortical lesions (Polyak, 1957) suggests that geniculate-ganglion cells and also cortical cells having adjacent or overlapping receptive fields must be closely adjacent. This provides some further justification for using large electrodes which pick up the summed effects from many cells. Direct mapping of receptive fields of adjacent cortical nerve cells has been achieved on one occasion. All the fields seen were overlapping. In the monkey's lateral geniculate body such receptive fields have also been found to overlap (P. M. Daniel, K. N. Seneviratne and D. Whitteridge, unpublished).

The errors involved in the estimation of magnification factors are not negligible. Measurement of the distance between points in the visual field has an error of 10 % at least, and measurement of the distance between

corresponding points on the cortex has difficulties arising from uncertainties due to leading from different points in the thickness of the cortex, especially when this has been crossed obliquely. There are also errors in measurement of sections caused by unequal shrinkage. Nevertheless, the points obtained make it obvious that there is a relationship between cortical magnification and eccentricity (Text-fig. 4). Talbot & Marshall (1941) suggest in their text that this is a discontinuous relation but their Fig. 5 makes it clear that the relation is a continuous one.

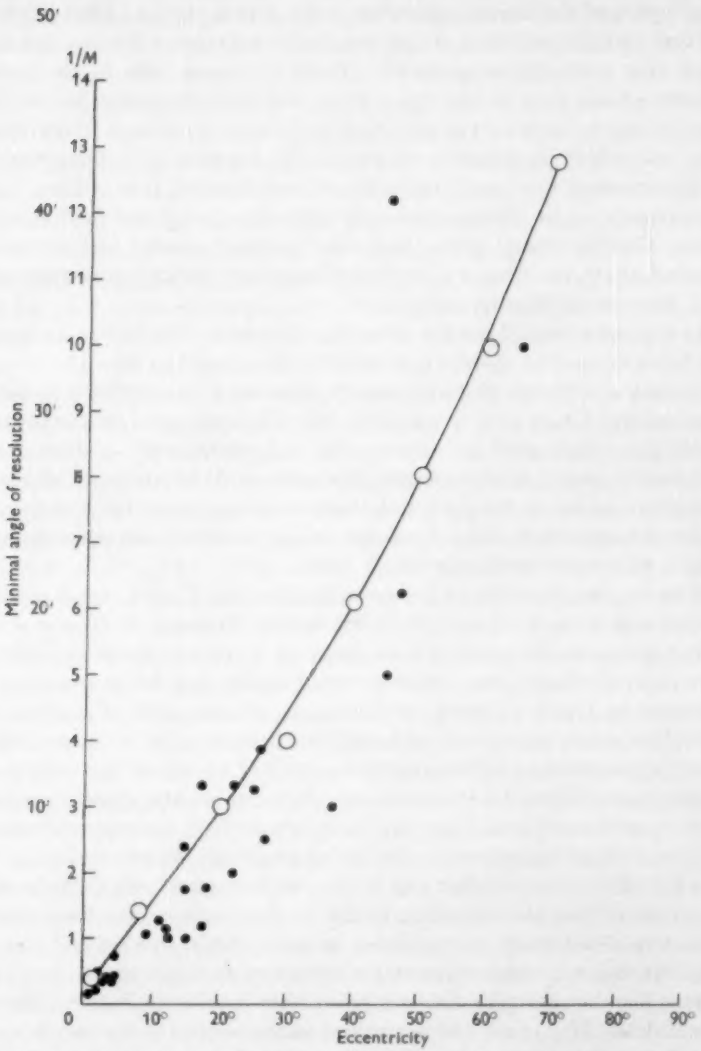
The only assumption which is required in order to calculate the area of the visual receptive field from measurements of cortical magnification is that the magnification depends only upon the distance from the fovea and, at any point, is the same for all directions. If all the data are grouped into six sectors each of 30°, as in Text-fig. 4, it seems that there is no appreciable difference between them. However, in the frog, there are large differences between magnification factors measured along horizontal and vertical meridians (Jacobson, 1960) and in the pigeon there is an area of increased magnification corresponding to the anterior end of the horizontal meridian (D. Whitteridge, unpublished observations).

In the monkey, differences in the shape of the visual area of the cortex between the scheme suggested here and the drawing of Polyak (1957, Fig. 259) are not very great. The foveal end of the visual area is, we think, more pointed and the peripheral end more rounded, and the semicircles of latitude appear in a surface projection as straight lines at right angles to the meridians. The boundary of the figure is formed almost entirely by the vertical meridian and the semicircle of latitude for 90° is reduced to a very small strip, in which our model differs considerably from Polyak's drawing.

Some attempt to relate minimal angle of resolution and the distribution of retinal ganglion cells in man has already been made by Weymouth (1958), who points out that both fall off linearly with eccentricity for 30°. There are, unfortunately, no reliable ganglion-cell counts for the peripheral retina. Before we can provide an anatomical basis for the minimal angle of resolution from the retina to the cortex, we need to know if there is multiplication or reduction of cell paths in the lateral geniculate body, and how axons from the lateral geniculate body are distributed to the cortex.

On the first point the data are probably insufficient. Brindley (1960) has quoted figures which suggest that there is multiplication of paths at the lateral geniculate in the monkey and even greater multiplication in the cat, but, on the contrary, a reduction in paths in man and in the rat. Unfortunately, estimates by different authors for the number of cells in the same structure do not agree closely.

Data are less incomplete for the monkey than for other animals. There are 1,210,000 nerve fibres in the optic tract (Bruesch & Arey, 1942) and




Text-fig. 8. The minimal angle of resolution for man (Weymouth, 1958), ○; and values for the reciprocal of the magnification factor for the monkey, ●.

1,590,000 cells in the small-celled layers and 209,000 cells in the large-celled layers of the lateral geniculate body (Clark, 1941). After allowing for fibres in the optic tract which run to the superior colliculus and elsewhere, this certainly suggests that there are more cells in the lateral geniculate body than in the optic tract, and that there may be twice as many. Glees & Clark (1941) have observed single optic-tract fibres ending on up to 5 cells in the lateral geniculate body, but they do not say whether this observation was made on cells at the posterior pole, which is the macular area, or at the anterior end where the peripheral field is represented. On the crucial point, therefore, whether central and peripheral fibres behave in the same way or in different ways at the lateral geniculate body, there is insufficient evidence.

For the optic radiations the situation is clearer. Clark (1941) suggests that there is an even distribution of 1350 fibres/mm$^2$ to area 17.

Sections of different parts of area 17 are remarkably uniform in appearance, though Chow *et al.* (1950) find 148 cells/0·001 mm$^3$ on the postero-lateral (macular) area as against 178 cells/0·001 mm$^3$ in the medial (peripheral) areas. Unfortunately the area of distribution of dendrites from single axons of the optic radiation is not known in the monkey. In the cat this extends to 650 $\mu$ diameter, so that one axon can make synaptic contact with some 5000 cells (Sholl, 1956).

From our own experimental data on the monkey, 1° at the fovea occupies about 6 mm linearly on area 17 of the cortex (Text-fig. 4). This is a considerably smaller figure than that given by Talbot & Marshall (1941) of 2′/mm, or 30 mm/degree. Central visual acuity has been measured for macaques by Grether (1941), who gives a minimal angle of resolution of 0·67′. This must correspond to a cortical distance of 67 $\mu$ in the area of foveal representation, or the distance occupied by about five cells in the densest part of layer IV of the cortex. So far there are no data available for the peripheral visual acuity in the monkey. It is, however, interesting to observe that magnification in the monkey and visual acuity in man both fall off in a very similar way in the peripheral field. In Text-fig. 8 the reciprocal of the magnification factor in the monkey has been plotted against minimal angle of resolution in man. While this way of plotting magnification minimizes the scatter of values at small angles and exaggerates it at large angles, there is reasonable agreement between the two sets of data. If, in fact, the peripheral visual acuity in the monkey does fall off as it does in man, it would follow that the minimal angle of resolution would correspond to about 67 $\mu$ on the cortex of the monkey, both for the foveal area and for the extreme periphery. Presumably two peaks of excitation would have to be separated by this distance and by a corresponding number of cortical cells for them to give rise to separate sensations.

Although the essential information on the magnification factor in man is lacking, there are interesting points of comparison. The total area of the calcarine cortex is about 3000 mm², or about twice that of the monkey, but the cell density is not greater than half (Shariff, 1953). This is probably related to the greater number of fibre connexions per unit area (cf. Wright, 1934). The shape of area 17 is probably similar to that in the monkey, though the folding of the cortex is very different. In transverse serial sections the foveal area is difficult to deal with and seems sometimes to have been omitted in studies of the human visual cortex. This is clear in one of the series of occipital lobes measured by Filimonoff (1933), where an apparently isolated sector of area 17 appears on the lateral surface about 1 cm anterior to the posterior pole. It is also clear from Filimonoff's very careful work that a vertical fold similar to that seen in the monkey does occur in man in one brain in three or four. There is, however, usually longitudinal folding on both sides of the vertical fold. In the commoner arrangement the longitudinal fold forming the calcarine fissure runs almost from end to end of area 17 and smaller folds both parallel and transverse are to be found in the depths of the calcarine fissure.

Recently it has been customary to minimize the importance of precise localization in the primary sensory areas. This has arisen partly from experiments of Lashley (1939) on the surviving ability of rats to acquire visual discrimination when all but a remnant of the visual cortex containing 700 geniculostriate cells in 1·5 mm² of cortex had been removed. This has been generalized into the statement that the visual area as a whole has some slight 'action propre', but not its parts (Boring, 1960). Insufficient attention seems to have been paid in interpreting these experiments to the rat's poor visual acuity, and the extent to which a very restricted visual field can be used by moving the head. The conclusions of Doty (1958) that localization in the cat's visual cortex was not precise, apply to the later cortical waves and not to the earlier responses, which are sharply defined and can be mapped in a point to point manner (D. Whitteridge, unpublished observations).

On the other hand, the classical observations of Holmes (1919) showed that sharply defined cortical lesions produce sharply defined scotomata with edges separating areas of normal acuity from areas of total blindness. The work of Hubel & Wiesel (1959) suggests that point-to-point representation on the cortex is an essential preliminary to the analysis of patterns by selective activation of cortical cells. According to Maturana, Lettvin, McCulloch & Pitts (1960) the corresponding process in the frog's tectum is carried out largely by retinal cells specifically sensitive to particular contours, but again a point-to-point representation on the corresponding surface in the central nervous system is present. In addition, more diffuse

mechanisms seem to be involved in the cortical responses of longer latency, but little is yet known of their afferent paths.

## SUMMARY

1. The representation of the visual field on the calcarine cortex in baboons and macaques has been determined by means of long steel electrodes of less than 10 $\mu$ tip and small visual stimuli subtending 10′ of arc at the eye.

2. The magnification factor, i.e. the linear extent of cortex concerned with each degree of visual field, has been determined. At the foveal region the minimal angle of resolution occupies an area of the cortex of about 67 $\mu$ in diameter.

3. The magnification factor appears to be the same for all points on a semicircle of latitude, and for small angles of displacement in the visual field it appears to be independent of the direction in which the displacement is taken.

4. From these data a surface can be calculated which can be folded to give a close approximation to the size and folding of the visual cortex.

5. The applicability of these results to man is discussed.

We are deeply indebted to Mr David Kendall of Magdalen College, Oxford, for suggesting the method of geometrical transformation and to Professor R. B. Fisher for advice on the treatment of the data. Part of the work was assisted by a grant from the Nuffield Foundation to Professor P. M. Daniel. We are also indebted to the Ross Foundation, Edinburgh, for the provision of a perimeter, to the Henderson Trust for the provision of some of the monkeys, and to Mr W. T. S. Austin and Mr R. Beesley for enthusiastic help.

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## EXPLANATION OF PLATE

A parasagittal section 100 $\mu$ thick of the baboon's occipital cortex 4 mm from the mid line. Five needle tracks are visible, and are very nearly in the plane of the section. Weil's stain. Scale in mm.

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# CHEMORECEPTOR ACTIVITY OF THE CAROTID BODY OF THE CAT

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The chemoreceptor activity of the carotid body of the cat has been extensively investigated both in the animal and in the organ perfused *in situ* (Heymans & Neil, 1958). It is generally accepted that hypoxia and hypercapnia increase the frequency of chemoreceptor discharges. However, there has been controversy concerning the threshold of these receptors to $O_2$ and $CO_2$ tensions and the extent to which afferent-discharge frequency is changed when the organ is exposed to different gas mixtures.

Comroe & Schmidt (1938) found that $O_2$ had to be lowered to 50 mm Hg in the perfused organ before the receptors reflexly modified respiration. However, von Euler, Liljestrand & Zotterman (1939) and Witzleb, Bartels, Budde & Mochizucki (1955) recorded chemoreceptor discharges from the carotid nerve when the blood $O_2$ tension was above 100 mm Hg. The role played by haemoglobin-carried oxygen has also concerned a number of investigators, particularly since the $O_2$-tension changes that greatly modify the frequency of the sensory discharges (below 100 mm Hg) also fall within the steep portion of Hb–$HbO_2$ dissociation curve. Von Euler *et al.* (1939) and Alvarez-Buylla (1951) suggested that chemoreceptor discharge is determined by blood oxygen saturation, discharge beginning when $HbO_2$ saturation falls below 96%. However, other evidence does not favour their view, since destruction of the $O_2$-carrying capacity of Hb by CO does not affect chemoreceptor responses to changes in $O_2$ tension (Comroe & Schmidt, 1938; Duke, Green & Neil, 1952).

Samaan & Stella (1935) found that $CO_2$ tension in the blood had to be raised above 32 mm Hg to activate carotid-body chemoreceptors. Von Euler *et al.* (1939) have reported that chemoreceptors start discharging when the $CO_2$ tension is raised to 30 mm Hg, whereas Bartels & Witzleb (1956) have obtained threshold discharges at $CO_2$ tensions below 30 mm Hg.

Experiments were performed in the whole animal to define the range of chemoreceptor response to variations in the inspired $O_2$ and $CO_2$. Results

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presented below indicate that chemoreceptors may be active even when the animal breathes pure oxygen. Their discharge increases with reduction of the inspired $O_2$, this effect apparently being dependent on tension rather than on $O_2$-content of the blood. Some chemoreceptors show activity at alveolar $pCO_2$ levels below 28 mm Hg and some chemoreceptor fibres respond both to hypoxia and to hypercapnia. A preliminary report has already been published (Eyzaguirre & Lewin, 1960).

## METHODS

Cats were anaesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories). The neck was opened in order to expose the carotid nerve, which was severed as far centrally as possible. Exposed tissues were covered with warm light paraffin oil and the nerve was laid on fine platinum-iridium electrodes. Sensory discharges were recorded by means of a.c. amplifiers, while the signals were fed to a double-beam oscilloscope for display. In addition, the output of the amplifier system was connected to an electronic counter and printer (CMC; Computer Measurements Co.) which recorded the number of impulses for 1 sec once every second or once every 10 or 15 sec during the experiment. When carotid nerve filaments containing only chemoreceptor fibres were desired, these were obtained by division of the nerve. In all experiments the other carotid nerve and both aortic nerves were severed before recording, to decrease reflex blood-pressure changes during hypoxia or hypercapnia. The nerves connecting the glomus with the superior cervical ganglion were also severed.

In all experiments a femoral vein was cannulated for injections. Flaxedil (gallamine triethiodide; American Cyanamid Co) 1–2 mg/kg was given intravenously to paralyse spontaneous respiration. This effect of Flaxedil lasted for 60–90 min. After this period of time the foot of the animal was squeezed to provoke a withdrawal reflex. If this reflex occurred, sodium pentobarbital (15 mg/kg) was given intraperitoneally. When withdrawal reflexes could not be elicited any more, Flaxedil 1–2 mg/kg was injected again intravenously to produce complete respiratory paralysis. These procedures were repeated as required.

The trachea was cannulated and connected to a demand valve respirator (Ensco). The instrument allowed controlled changes of inspiratory pressure (and hence of tidal volume) and of the duration of inspiration and expiration. The input of the respirator was connected to cylinders containing known gas mixtures under pressure, and by a suitable arrangement of valves it was possible to switch rapidly from one gas mixture to another. The expired gases were passively expelled from the system through a valve in the respirator. The dead space between the respirator and the trachea was less than 20 ml. Ventilation rates varied from 15 to 24 breaths/min in different experiments, with a ratio of inspiratory–expiratory durations of from 1:3 to 1:1·5. An 18-gauge needle was inserted into the tracheal cannula and in turn connected to a microcatheter cell of an infra-red $CO_2$ analyser (Beckman) for continuous monitoring of the alveolar (end-tidal) $CO_2$. The output of the $CO_2$ analyser was connected to one channel of a Grass polygraph, while the other channel of the polygraph recorded blood pressure from the femoral artery through a P 23 AC Statham pressure gauge. The rectal temperature of the animal was kept at 37–38° C. The experiments were conducted at barometric pressures of from 638·0 to 645·6 mm Hg.

## RESULTS

Nerve discharges in the carotid nerve were identified as coming from pressoreceptors whenever their discharge occurred in bursts synchronous with the pulse. Chemoreceptor discharges were identified as those impulses

having no relation with the pulse, whose number increased after turning off the respirator. The counter was gated to record only nerve impulses which were clearly above the amplifier noise. Since in most experiments there was obvious contamination of chemoreceptor discharges by pressoreceptor impulses, blood pressure was measured throughout the experiment in all cases. Pressoreceptor contamination produced relatively large frequency readings (number of impulses/unit time) and probably a non-linear discharge frequency because of overlap and summation of sensory discharges; but in spite of these limitations of the method the frequency curves are felt to provide a fairly good index of events occurring at the sensory receptors during their exposure to different concentrations of $O_2$ or $CO_2$.

*Effects produced by changes in $O_2$ content of the inspired gas*

In this experimental series ventilation was kept as constant as possible while the $O_2$ content of the inspired gas mixture was varied. Gas mixtures containing 10, 20, 30, and 50 % $O_2$ in $N_2$ as well as 100 % $O_2$ were given to the animal in different sequences while nerve impulses were recorded from the carotid nerve. Figure 1 is taken from an experiment which illustrates the variations in carotid-nerve discharge produced by changes in the inhaled oxygen mixture. The upper part of the illustration shows a record obtained during a 20 min period. The mean blood pressure was stable at about 110 mm Hg and the alveolar $pCO_2$ fluctuated between 28·5 and 30·2 mm Hg throughout that period. A base-line discharge of about 120/sec occurred while the animal inhaled 50 % $O_2$ in $N_2$. The frequency increased to about 310/sec in about 3·5 min when the gas mixture was replaced by 20 % $O_2$ in $N_2$, following which the discharge declined slightly and became stable at about 280/sec. Several minutes later the 20 % $O_2$ was replaced by 10 % $O_2$ in $N_2$, and the discharge frequency increased to about 520/sec in about 4 min; it became relatively stable at this new level. At the downward arrow (Fig. 1) the gas mixture was again replaced by 20 % $O_2$ and the frequency declined to about 200/sec in about 3 min. Several minutes later (not shown) the frequency rose to a value similar to that previously obtained during inhalation of 20 % $O_2$. The lower part of the illustration shows measurements taken from the same preparation 1 hr later. During this series the mean blood pressure fluctuated between 104·6 and 106·6 mm Hg and the alveolar $pCO_2$ from 28·5 to 29·7 mm Hg. With the animal breathing 20 % $O_2$ the impulse frequency was about 220/sec. About 1 min after 20 % $O_2$ was replaced by 100 % $O_2$ the discharge declined to about 50/sec. Four minutes later 100 % $O_2$ was replaced by 50 % $O_2$ in $N_2$; the discharge frequency then slowly increased to about 100/sec. Finally, 100 % $O_2$ was given again and discharges declined to about 60 sec.

The experiment just described shows that the maximal effect on chemoreceptor discharge produced by reduction of the inhaled oxygen is reached several minutes after a change in the inspired gas. The slowness of this effect may be due to a relatively slow washing out of the residual lung volume and/or to a genuinely slow chemoreceptor activation (Eyzaguirre & Lewin, 1961).

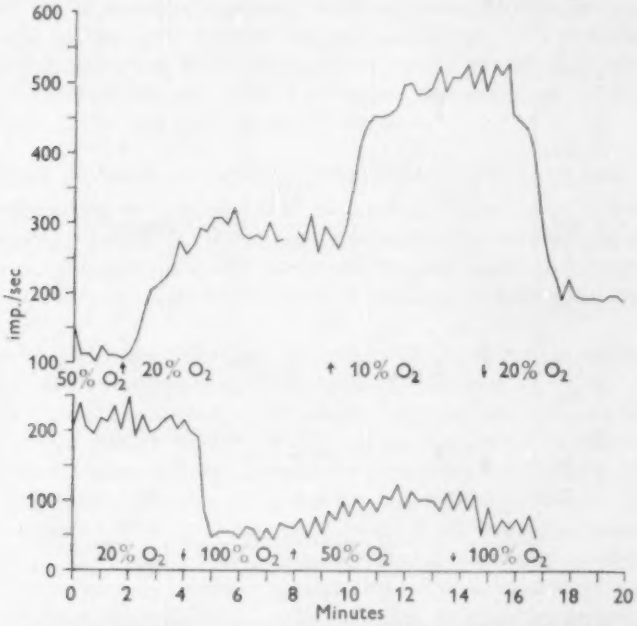


Fig. 1. Changes in carotid-nerve impulses during inhalation of different oxygen mixtures. Recording from the whole carotid nerve. Discharges mainly from chemoreceptor fibre activity; some contamination from pressoreceptor discharges. Mean blood pressure, 104·6–106·6 mm Hg. Alveolar $pCO_2$, 28·5–29·7 mm Hg throughout series. Lower trace 1 hr after upper trace.

A more accurate estimate of the range in which chemoreceptors respond to different concentrations of inhaled oxygen was obtained in another series. The animal inhaled 100% $O_2$, and once sensory discharges had become stable the average frequency per second was calculated. The same procedure was repeated after inhalation of 50, 30, 20 and 10% $O_2$ in $N_2$. Figure 2 illustrates such an experiment. The upper curve shows the relationship between impulses in the carotid-nerve discharge and concentration of $O_2$ in the inhaled gas. As the concentration of the inhaled oxygen was reduced, the increase in frequency of individual units and

recruitment of previously silent fibres could be identified on the oscilloscope screen. At all times during the experiment the blood pressure and alveolar $pCO_2$ were measured (middle and lower curves in the illustration).

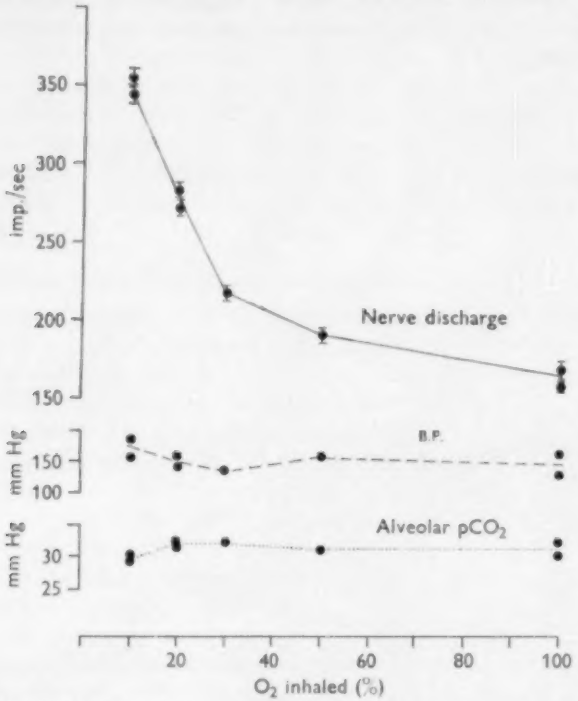


Fig. 2. Relationship between concentration of inhaled $O_2$ and carotid-nerve impulses. Upper curve, average frequency during steady state $\pm$S.D. Middle curve, blood pressure. Lower curve, alveolar $pCO_2$.

The experiments illustrated in Figs. 1 and 2 show that a small but significant increase in chemoreceptor discharge may occur when pure oxygen is replaced by 50 % $O_2$ as inspired gas. This effect, however, does not always occur; a number of preparations fail to show a clear difference in chemoreceptor activity between these concentrations of inspired oxygen. The increase in chemoreceptor discharge is always large when the inspired gas is reduced from 100 or 50 % $O_2$ to 30, 20 and 10 % $O_2$. These observations touch on the point concerning the threshold of chemoreceptors to oxygen. The non-pulsatile discharge, presumably coming from chemoreceptors, does not stop completely when the animal breathes pure oxygen; it may be clearly identified on the oscilloscope screen. Moreover, chemo-

receptor discharges present during inhalation of gas mixtures containing more oxygen than room air need not result from an action by $CO_2$ as suggested by von Euler *et al.* (1939). Indeed, when $O_2$ mixtures are changed from 100 to 30 % $O_2$ and the alveolar $pCO_2$ remains unchanged the increment in chemoreceptor discharges is still considerable (Fig. 2). Another important point is that inhalation of different $O_2$ mixtures, in a range wherein Hb is nearly completely saturated with oxygen, still produce variations in the carotid-nerve impulse discharge. This indicates that oxygen tension rather than $HbO_2$ saturation of the blood is the factor responsible for stimulation of these receptors, as was suggested by Comroe & Schmidt (1938) and by Duke *et al.* (1952).

*Effects of changes in ventilation; the effects of $O_2$ and $CO_2$ tensions*

The preceding section described changes in chemoreceptor discharges produced by inhalation of different $O_2$ mixtures under constant ventilation. In this section changes in chemoreceptor impulse activity during inhalation of different $O_2$ mixtures obtained at different alveolar $pCO_2$ levels will be described.

Alveolar $pCO_2$ was changed by increasing or reducing the inflation pressure of the respirator with the animal breathing 100 % $O_2$. Once a given $CO_2$ level was obtained, the inspired oxygen was reduced, in steps, to 50, 20 and finally 10 % $O_2$ in $N_2$. At each $O_2$ level discharges were allowed to stabilize and the average frequency was calculated. After the end of a series the alveolar $pCO_2$ was set at a different level and the procedure repeated. The alveolar $pCO_2$ levels studied ranged from 10 to 60 mm Hg. It must be said beforehand that a reduction in ventilation may produce not only hypercapnia but also a decreased arterial $pO_2$, even if the inhaled $O_2$ is maintained constant. But, as is shown below, hypercapnic effects alone may be obtained if the inhaled mixture contains 50 % or more oxygen, while at lower levels of $O_2$ inhalation a clear combination of hypoxia and hypercapnia occurs during changes in ventilation. Figure 3, taken from such an experiment, shows the relations between alveolar $pCO_2$, concentration of inspired $O_2$ and carotid-nerve discharge frequency, plotted in an isometric diagram. In this experiment contamination from pressoreceptor discharges was negligible. From the illustration one may see that the lower two oxygen–impulse curves, recorded at alveolar $pCO_2$ levels below 30 mm Hg, are similar to that presented in Fig. 2. When the $CO_2$ level was raised above 40 mm Hg (upper three oxygen–impulse curves) the curves started at a higher frequency level and tended to become steeper when the inspired $O_2$ was reduced from 50 to 10 % $O_2$. At these high $pCO_2$ levels reduction of the inspired oxygen from 20 to 10 % $O_2$ did not increase chemoreceptor discharge, since the frequency curves had a tendency to

level off. The $CO_2$–impulse curves obtained during inhalation of 100 or 50 % $O_2$ are similar. They are S-shaped with a tendency to reach their peak once alveolar $pCO_2$ rises above 40 mm Hg. The $CO_2$–impulse curve obtained during inhalation of 20 % $O_2$ had a similar shape and also a tendency to reach maximal discharge at the same alveolar $pCO_2$ levels, although the maximal absolute level was higher. Finally, the $CO_2$–impulse curve constructed during inhalation of 10 % $O_2$ reached its peak when $pCO_2$ was raised near 30 mm Hg.

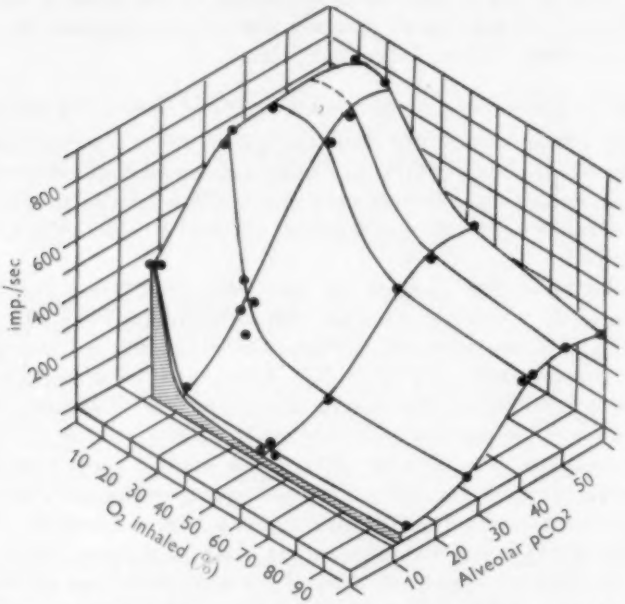


Fig. 3. Isometric diagram representing changes in carotid nerve impulses during inhalation of different $O_2$ mixtures at various ventilation levels. *X* axis, alveolar $pCO_2$; *Y* axis, concentration of inhaled oxygen; *Z* axis, frequency of carotid-nerve impulses; discharges mainly from chemoreceptors. Mean blood pressure 140–170 mm Hg throughout the series. Oxygen–impulse curves obtained during inhalation of $O_2$ (100–10 %) at different $pCO_2$ levels. $CO_2$–impulse curves represent impulses obtained at different $pCO_2$ levels during inhalation of pure oxygen, and of 50, 20 and 10 % $O_2$ in $N_2$ (see text).

Inspection of the isometric surface shows that chemoreceptors are capable of responding to low $CO_2$ tensions (27–28 mm Hg or less), as was suggested by Bartels & Witzleb (1956). Increasing $pCO_2$ levels (during inhalation of more than 30 % $O_2$) determined an elevation of the isometric surface which may be entirely due to hypercapnic effects. On the other hand, the steep

incline shown at the left of the surface is probably produced by hypoxia and by a combination of hypoxia and hypercapnia.

The maximal response of the chemoreceptors to $CO_2$ appears when $pCO_2$ is raised above 40 mm Hg. This response, however, appears at a lower discharge level when the animal inhales 50–100 % $O_2$ than during inspiration of 20–10 % $O_2$. The additional discharges in the latter case are produced by recruitment of new units (identified on the oscilloscope screen) and probably by an increase in the frequency of individual chemoreceptor fibres (see next section). The upper left edge of the isometric surface shows that discharges obtained during inhalation of 10 % $O_2$ may still be increased if alveolar $pCO_2$ is increased up to about 30 mm Hg. Further increase in $CO_2$ does not produce greater frequency of chemoreceptor discharge; the receptors seem to have reached total 'saturation'. In conclusion, it seems that there is a definite interaction in the response of chemoreceptors to $O_2$ and $CO_2$. A similar conclusion has been reached recently by Joels & Neil (1961), using the perfused carotid body of the cat.

*The response of single chemoreceptor fibres to hypoxia and to hypercapnia*

Results presented in the preceding section suggested the possibility that single chemoreceptor fibres would change the frequency of their discharge under the influence of both low oxygen and high carbon-dioxide tensions. In experiments designed to test this possibility, single fibres were dissected from carotid nerve filaments and tested for chemoreceptor activity as indicated previously. The animal was made to inhale gas mixtures containing different concentrations of $O_2$ or $CO_2$. Figure 4 illustrates such an experiment. The animal was made to inhale either 100 % $O_2$, 20 % $O_2$ in $N_2$ or 6 % $CO_2$ in $O_2$. In all series the counter was set to count impulses at 1 sec intervals once the discharge had become stable at each new level. Figure 4A shows that during inhalation of 100 % $O_2$ aperiodic discharges occurred and during long intervals of time no impulses were recorded. Figure 4B shows another run in the same preparation, but this time the animal was inhaling 6 % $CO_2$ in $O_2$. The discharge was still irregular but the silent periods were shorter than in A. Figure 4C illustrates another run after returning to 100 % $O_2$, and the discharge pattern was similar to that recorded in A. Figure 4D shows the discharge obtained during inhalation of 20 % $O_2$. In this case more impulses per unit time were counted and discharges occurred during each of the measured intervals. Figure 4E shows results similar to those illustrated in B. In this experiment the alveolar $pCO_2$ was 42–46 mm Hg during inhalation of 100 or of 20 % $O_2$ and it rose to 53–57 mm Hg during inhalation of 6 % $CO_2$.

The experiment illustrated in Fig. 4 shows that the discharge of single chemoreceptor fibres is aperiodic, particularly during inhalation of pure

oxygen. Inhalation of 6 % $CO_2$ in $O_2$, which increases the alveolar $pCO_2$ to values where chemoreceptors respond maximally to $CO_2$, still produces an aperiodic discharge, although the silent periods between discharges are shorter than during inhalation of pure oxygen. Inhalation of 20 % $O_2$ instead of 6 % $CO_2$ in $O_2$ seems to be a more adequate stimulus to these receptors, since the discharge is more regular and it occurs at higher

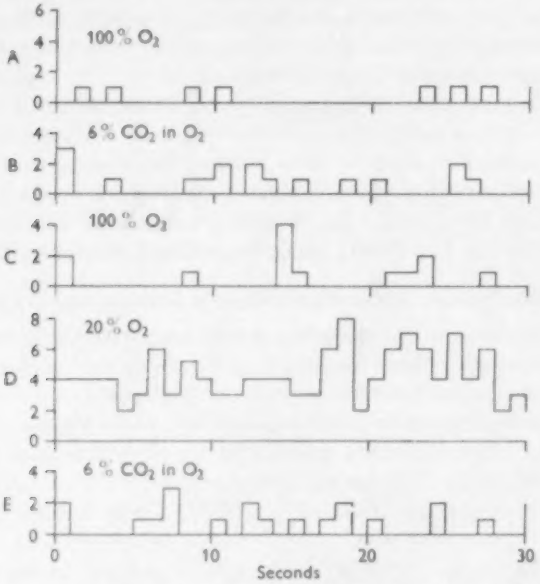


Fig. 4. Frequency changes in single chemoreceptor fibre during inhalation of different gas mixtures. Five runs in the same preparation (A, B, C, D and E), each run obtained after occurrence of steady state (see text). Alveolar $pCO_2$ 42–46 mm Hg during inhalation of pure oxygen or $O_2$ in $N_2$; 53–57 mm Hg during inhalation of 6 % $CO_2$ in $O_2$. Ordinate, frequency (imp./sec); abscissa, time.

frequency. This observation brings the chemoreceptors into line with other well known slowly-adapting receptors, where weak stimulation determines an irregular discharge; this irregularity tends to become less conspicuous when the stimulus is increased. A better view of events occurring in single chemoreceptor fibres during inhalation of 100 % $O_2$, 20 % $O_2$ or 6 % $CO_2$ in $O_2$, is presented in Fig. 5. The animal inspired a given gas mixture for several minutes; once discharges reached a steady state the counter was set to count the total number of impulses occurring during a period of 20 sec. After this period the gas mixture was changed and the procedure repeated. It is clear that fewer impulses were always obtained during

inhalation of pure oxygen. The number of recorded impulses increased when 100 % $O_2$ was replaced by 6 % $CO_2$ in $O_2$; their number increased still further when 20 % $O_2$ was inspired.

The experiment just described shows that a single chemoreceptor fibre may respond both to low oxygen and high $CO_2$ tensions. Although only four fibres were analysed in this series, because of technical difficulties, it is suspected that non-specificity of chemoreceptor fibres to such stimulation is common. Joels & Neil (1961) have observed similar double effects in the perfused carotid body of the cat while recording from single chemoreceptor fibres.

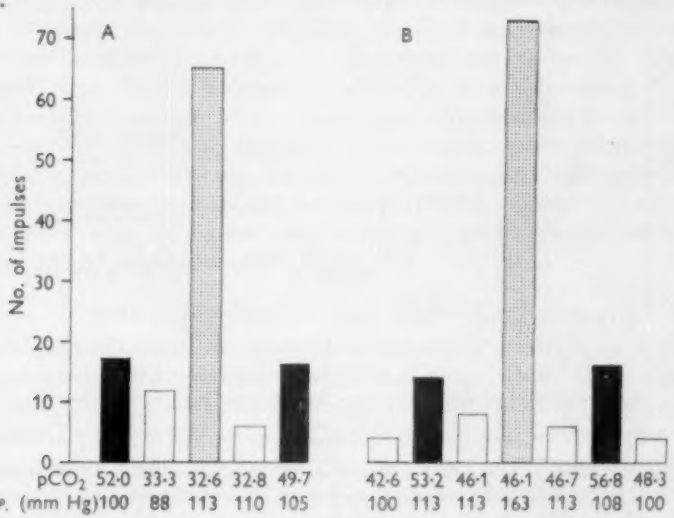


Fig. 5. Effects of changes in oxygen and $CO_2$ tension on single chemoreceptor-fibre discharge. A and B, two runs in the same preparation at 20 min interval. Bars represent number of impulses counted over a period of 20 sec during steady state. □ 100 % $O_2$; ▨ 20 % $O_2$ in $N_2$; ■ 6 % $CO_2$ in $O_2$.

*Effect of blood-pressure changes on chemoreceptor discharges*

It is well known that a decrease in systemic blood pressure produces an increase in chemoreceptor activity. In fact, Landgren & Neil (1951) have shown such an effect by bleeding an animal and lowering the mean blood pressure to about 50 mm Hg. However, no systematic studies relating blood-pressure changes and chemoreceptor discharges seem to be available.

The present series was designed to study under controlled conditions the effects of low blood pressure on chemoreceptor discharge. Figure 6 illustrates such an experiment. The cat was breathing air while carotid nerve impulses, blood pressure and alveolar $CO_2$ were simultaneously recorded.

As in other experiments, both aortic nerves, the other glossopharyngeal nerve and the ganglio-glomerular connexions were severed. The carotid nerve in this preparation yielded a discharge of some 350 imp./sec with a range between 300 and 400 imp./sec. The mean blood pressure of the animal ranged from 140 to 150 mm Hg, while alveolar $pCO_2$ was 25·1 mm Hg. Hexamethonium (hexamethylenebis(trimethylammonium chloride); Burroughs Wellcome) 5 mg/kg was given intravenously. One minute after the injection the nerve impulses rose to about 460/sec. At this

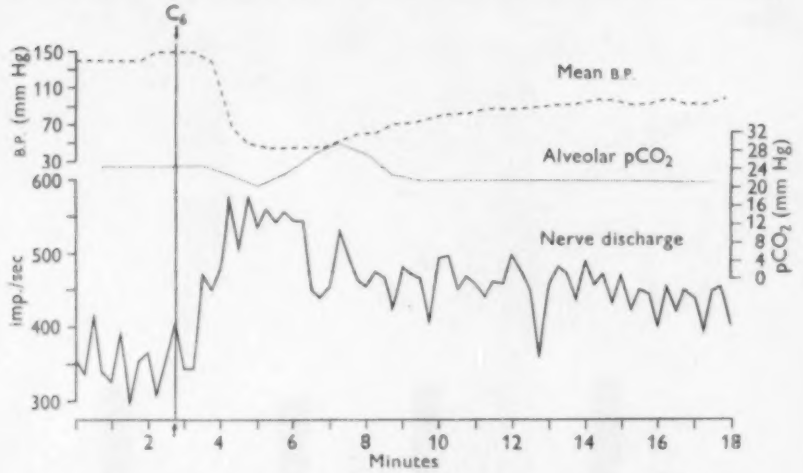


Fig. 6. Effect of systemic blood-pressure changes on carotid-nerve discharges. Vertical line indicates time of intravenous injection of hexamethonium 5 mg/kg.

point the blood pressure of the animal remained unchanged (140 mm Hg) and the alveolar $pCO_2$ was still 25·1 mm Hg. Thirty seconds later the impulses rose to 575/sec while blood pressure fell to 70 mm Hg and alveolar $pCO_2$ decreased to 21·6 mm Hg. Further lowering of blood pressure to 45 mm Hg caused no further change in the frequency of chemoreceptor discharges. At the latter blood-pressure level no nerve discharges synchronous with the pulse could be identified on the oscilloscope screen. Eight minutes after the injection the blood pressure rose to about 90 mm Hg but the discharge frequency was still about 450 imp./sec. Thirty minutes after the injection the preparation recovered to base-line levels (not illustrated).

The experiment just described shows that after a large dose of hexamethonium chemoreceptor discharges started to increase before any appreciable change in systemic blood pressure was detectable. This effect

may be due to an initial stimulant action of hexamethonium on the chemoreceptors, although the available evidence indicates that this drug depresses chemoreceptor discharges (Gollwitzer-Meier & Witzleb, 1953; Dontas & Nickerson, 1954). Consequently, further clarification of the early discharge produced by hexamethonium during hypotension is desirable. Some animals showed a great sensitivity in their chemoreceptor response to blood-pressure changes, since variations of the order of 20 mm Hg produced at times a marked change in chemoreceptor impulse traffic. Other preparations, on the other hand, were rather unresponsive to blood-pressure changes with regard to chemoreceptor discharges. The cause of these variations is unknown and they may depend on local circulatory conditions, which may vary from one animal to another (Chungcharoen, Daly & Schweitzer, 1952). In general, however, it was found that maintenance of a normal blood pressure was essential for chemoreceptor response to variations in the inhaled gases. Animals with low blood pressure (50–80 mm Hg) showed a dense carotid nerve discharge but were unresponsive to other forms of stimulation. However, Landgren & Neil (1951) have shown that chemoreceptor hypotensive discharge may be decreased by inhalation of 100 % $O_2$.

*Chemoreceptor discharges during carotid occlusion*

In the cat occlusion of the external carotid artery produced an appreciable increase in chemoreceptor discharges (Chungcharoen, Daly, Neil & Schweitzer, 1952). In order to study the temporal course of these events, experiments were performed in which the carotid and aortic nerves and the ganglio-glomerular connexions were previously cut; the animals were breathing 20 % $O_2$ under controlled ventilation. Such an experiment is illustrated in Fig. 7. A base-line discharge of about 185 imp./sec was recorded. At the upward arrow, flow in the ipsilateral carotid artery was interrupted and discharges rose to about 450 imp./sec in 25 sec. At this point pressoreceptors were silent. A few seconds later the clip was rapidly removed and discharges decreased to a minimum of 75 imp/sec in about 15 sec. Afterwards discharges came back to base-line levels in about 30 sec.

What is noteworthy in this experiment is that the effects produced by carotid occlusion develop relatively slowly (about 25 sec) and that discharges come back to base-line levels only after a period of depression. This undershoot may be due to a genuine off-effect, as it occurs in other receptor organs, or to reactive hyperaemia. Reflex vascular changes can be excluded, since the organ was isolated from all its nervous connexions. Carotid occlusion did not always produce such striking changes in chemoreceptor impulse discharge.

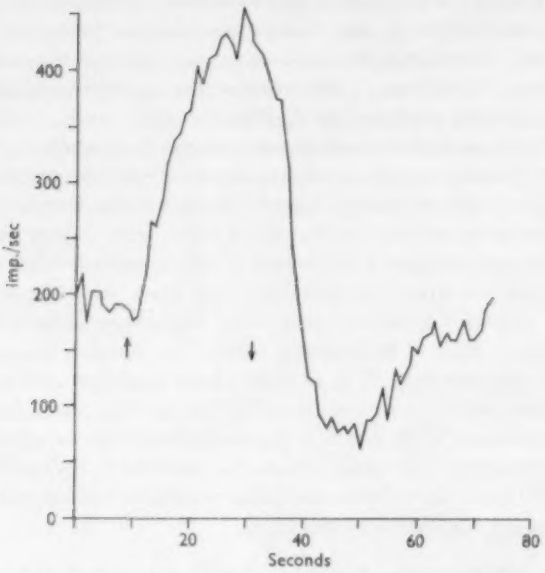


Fig. 7. Carotid-nerve impulses during carotid occlusion. Recording from whole carotid nerve. At 1st (upward) arrow, external carotid artery occluded; at 2nd (downward) arrow, clip removed. Note increase in chemoreceptor discharges and depression after removing clip.

## DISCUSSION

Carotid-body chemoreceptors discharge to a wide range of $O_2$ concentrations in the blood and this effect appears to be dependent on the oxygen tension of the blood rather than on the oxygen saturation of haemoglobin. That the latter is not the important factor in determining the discharge in the chemoreceptors has been suspected for some time (Duke *et al.* 1952). At first glance this may seem unexpected, since the oxygen consumption of the glomerular tissue is very high. But under normal circumstances the high $O_2$ consumption is compensated by a very rapid blood flow (2000 ml., 100 g/min, according to Daly, Lambertsen & Schweitzer, 1954). However, one cannot dismiss entirely some role of haemoglobin in the activity of these receptors, since it is a constant source of $O_2$ which provides an adequate gradient to a tissue which is really an oxygen sink. The oxygen-carrying capacity of haemoglobin may become a very important auxiliary if for some reason blood flow through the region is impaired, as it may occur during carotid occlusion or hypotension.

The question of the threshold of chemoreceptor fibres to oxygen and $CO_2$ tensions seems to be clearer now than it has been in the past. In fact,

some low-threshold units still discharge when ventilation is very high and the animal is inhaling pure oxygen. Other units are less sensitive and are activated only when the oxygen tension is drastically reduced. The same is true of units responding to $CO_2$ tension, since some fibres have a low threshold to $CO_2$ (27–28 mm Hg or less) while others need a higher $CO_2$ level to start discharging. In this connexion the higher threshold of respiratory chemoreflexes to low oxygen and high $CO_2$ have been somewhat puzzling; $O_2$ has to be lowered to 100 mm Hg and $CO_2$ increased to 37 mm Hg before detectable reflexes occur (cf. Heymans & Neil, 1958). But a close inspection of the $O_2$ and $CO_2$–impulse curves presented in Figs. 2 and 3 may give a clue to this puzzle. Oxygen in the inhaled mixture has to be reduced below 30 % $O_2$ before there is a steep increase in chemoreceptor discharge. It is possible that inhalation of mixtures richer in oxygen does not produce enough impulses to drive the respiratory centre. Likewise the S-shaped $CO_2$–impulse curves of Fig. 3 during inhalation of 20–100 % $O_2$ show a steep ascent between 30 and 40 mm Hg, and it is between these points that reflex driving of respiration becomes evident. Also, from Fig. 3 one would expect that reflex driving of respiration would occur at lower $CO_2$ levels when 10 % $O_2$ is inhaled.

One salient point is the fact that single chemoreceptor fibres can respond to changes in both $O_2$ and $CO_2$ tension. This is probably a common phenomenon, although the number of units sampled is small. It is not known, however, whether a single receptor site is non-specific or whether a single sensory fibre branches and innervates more than one receptor site specific either to hypoxia or to hypercapnia. Further work is necessary in order to elucidate this point.

In conclusion it may be stated that carotid-body chemoreceptors respond to $O_2$ and $CO_2$ tension changes and that their discharge frequency is modified by reduction of the oxygen tension and by an increase of the $CO_2$ tension. However, at present the mechanism of this action is not known; a number of transmitters, metabolic and pH changes have been suggested as responsible for stimulation of the chemoreceptors by a number of workers in the field (cf. Heymans & Neil, 1958). But it is felt that the evidence available does not at present allow us to advance a plausible hypothesis as to the mechanism of activation of these receptors or about the nature of a possible transmitter substance.

## SUMMARY

1. The carotid-body chemoreceptors respond to a wide range of oxygen changes. Inhalation of pure oxygen produces a low-frequency discharge in low-threshold chemoreceptors. Reduction of the inhaled oxygen

increases the frequency of firing of individual sensory units and recruitment of previously silent units occurs.

2. A reduction of the $O_2$ tension in the plasma seems to be the factor responsible for the activation of these receptors. Saturation of Hb by $O_2$ seems to play only an auxiliary role.

3. Changes in ventilation alter the impulse traffic of the carotid nerve. Some of these effects can be attributed to an action of $CO_2$. Chemoreceptors may be stimulated at alveolar $pCO_2$ levels of less than 28 mm Hg.

4. Single chemoreceptor fibres respond both to hypoxia and to hypercapnia. It is not known whether chemoreceptors are non-specific in their response to these types of stimulation or whether a single chemoreceptor fibre branches and innervates different receptor sites specific either to hypoxia or to hypercapnia.

5. A fall in blood pressure strongly stimulates these receptors. However, chemoreceptor discharges start to increase (after hexamethonium) before a general fall in blood pressure occurs.

6. Carotid occlusion may stimulate chemoreceptors. Release of the occlusion brings back discharges to base-line levels after a period of depression.

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# EFFECT OF DIFFERENT OXYGEN TENSIONS ON THE CAROTID BODY *IN VITRO*

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Several studies performed in the animal have indicated that carotid-body chemoreceptors are stimulated by a reduction of the oxygen tension the plasma rather than by a decrease in the oxygen saturation of haemoglobin. Comroe & Schmidt (1938) have been able to elicit respiratory reflexes during perfusion of the carotid body with blood equilibrated with carbon monoxide, in which the oxygen tension was low. Duke, Green & Neil (1952) have found that destruction of most of the oxygen-carrying capacity of haemoglobin by CO does not depress the response of these receptors to inhalation of low-oxygen gas mixtures.

The findings just described suggested the possibility of studying the response of carotid-body chemoreceptors to changes in $O_2$ tensions in preparations excised from the animal and placed in Locke's solution equilibrated with gas mixtures containing different amounts of oxygen. Results presented below indicate that the excised glomus and the carotid nerve survive for hours in such a medium and that chemoreceptors respond at least qualitatively to different oxygen tensions as they do in the animal (Eyzaguirre & Lewin, 1961).

Chemoreceptors discharge at low frequency when the bathing solution is equilibrated with 100–50 % $O_2$; their discharge increases to a maximum when the oxygen is reduced to 20–10 %. This reduction in $O_2$ usually produces a frequency change which consists of a high-frequency peak followed by 'adaptation' of the receptors. Under the conditions employed the changes in chemoreceptors frequency produced by changes in $O_2$ tension of the saline proceed relatively slowly. Also, chemoreceptor discharge is enhanced by an increase in saline temperature above 37° C. A preliminary report has already been published (Eyzaguirre & Lewin, 1960).

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## METHODS

The carotid body and its own nerve, together with a stretch of carotid artery, were removed from cats anaesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories). Some animals received 10 mg heparin (Liquemin, Roche) 15–30 min before excision of the organ. Connective tissues surrounding the glomus were removed by dissection. Once cleaned the preparation was mounted in a small transparent chamber through which flowed modified Locke's solution (NaCl 9·0, KCl 0·42, and $CaCl_2$ 0·24 g/l.) previously mixed with glucose 1·0 g/l. The pH of the Locke's solution (7·4–7·45) was adjusted by means of Tris-HCl (tris(hydroxymethyl)-aminomethane) and its temperature was kept at or below 37° C.

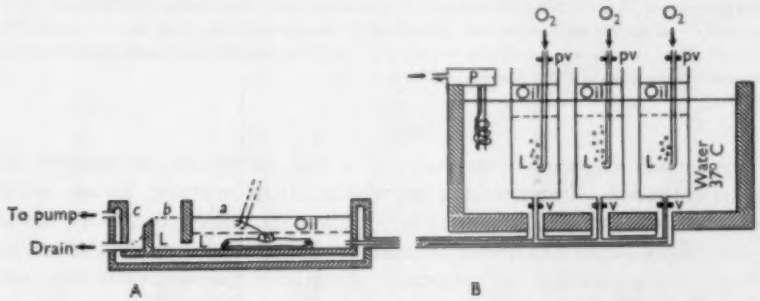


Fig. 1. Diagram of experimental arrangement. A, experimental chamber with three compartments, *a*, *b* and *c*; *a* contains carotid body in Locke's solution (L) covered by paraffin oil where nerve is on recording electrodes; *b* communicates with *a* through small tunnel and contains saline from communicating vessels system; *c*, overflow chamber separated from *b* by partition. Saline is drained from *c* by suction pump. Tunnel bored around compartments allows flow of warm water coming from B. B, large tank filled with water at or below 37° C; temperature controlled by stirrer-circulator P which also induces flow of water through chamber A. In the tank, three vessels containing Locke's solution (L) equilibrated with different $O_2$ mixtures and covered by thick layer of mineral oil. Oxygen flow controlled by valves pv; saline outflow controlled by valves v (see text).

Figure 1 illustrates the experimental arrangement. A shows the experimental chamber machined from a Plexiglass block. The interior of the chamber had three compartments; compartment *a* contained the preparation and communicated with *b* through a small tunnel bored under the partition separating *a* and *b*. This arrangement allowed the flow of Locke's solution (at 3·1–9·0 ml./min in different experiments) from under a layer of paraffin oil which remained undisturbed. Compartment *b* was separated from *c* by a slanted top partition which allowed saline to overflow into *c*, from where it was drained by a suction pump. The nerve was lifted into the oil in *a*, for recording of action potentials, which were fed to an oscilloscope, counter and printer (see Eyzaguirre & Lewin, 1961). Pressoreceptor fibres were eliminated by keeping the artery unstretched and by removing most of the adventitia (Alvarez-Buylla, 1954). B shows a large tank filled with water at temperatures thermostatically controlled by a Tecam circulator and stirrer (P) which also circulated the water through the walls of the experimental chamber (*a*). Inside the tank were three vessels (2000 ml.) containing Locke's solution (L), covered by a thick layer of paraffin oil. An oxygen mixture containing either pure oxygen or 50, 20, 10 or 5 % $O_2$ in $N_2$ was vigorously bubbled into each vessel, beginning at least 90 min before, and continuing throughout, the experi-

ment. The vessel's outflow was controlled by valve v, placed between the Locke reservoir and the experimental chamber. The connecting tube was enclosed in a larger diameter tube through which the warm water of the tank reached the experimental chamber.

On changing to a new solution by opening valve v, a dead space of 13 ml. had to be washed out before the new solution reached the preparation. Also, some mixing occurred in this dead space when solutions were exchanged. The time course of solution exchange was determined by placing a creatinine dye in one of the reservoir vessels and distilled water in another. The dye was allowed to flow through the experimental chamber for several minutes and then a 1 ml. sample was drawn. The flow of dye solution was interrupted and distilled water was allowed to flow. Samples (1 ml.) were taken once very 60 sec from the experimental chamber for a period of 10 min. This procedure was repeated several times at different rates of flow (3·2–9·0 ml./min) in this and in the reverse order. The samples were analysed in a photocolorimeter and dye-dilution or dye-concentration curves obtained. Some of these curves will be shown in the Results. The experiments were performed at barometric pressures of from 638·0 to 645·6 mm Hg.

## RESULTS

A successful experiment depended to a large extent on the anatomy of the preparation. Preparations in which the glomerular tissues were circumscribed and surrounded by a moderate amount of connective tissue yielded more consistent results than others in which glomerular tissue was diffuse and enveloped by abundant connective tissue. Also, previous administration of heparin to the animal usually resulted in lower frequency of discharges during exposure of the glomus to saline equilibrated with pure oxygen. Such preparations behaved more normally than others. In all probability heparin prevented intraglomerular clotting, thus providing a more permeable pathway for oxygen diffusion to the more deeply-placed glomerular tissues.

The solutions employed did not contain $CO_2$, which was purposely excluded since responses produced only by changes in $O_2$ tensions were sought. In this respect $CO_2$ exclusion was justified, since $O_2$ and $CO_2$ interact on these receptors (Joels & Neil, 1961; Eyzaguirre & Lewin, 1961). Lack of $CO_2$ may have had deleterious effects on the chemoreceptors (cf. Lorente de Nó, 1947) but the preparation could be used for periods longer than 10 hr and seemed to be maintained in relatively good condition. However, glomerular responses to lack of oxygen suffered a decline (in terms of changes in impulse frequency to different oxygen tensions) during the first 5 hr after excision of the organ, to reach a lower but relatively stable level thereafter. Results presented below were obtained during the initial period.

### *Chemoreceptor discharges at different oxygen tensions*

Chemoreceptors discharged at low frequencies when the flowing solution was equilibrated with 100–50 % $O_2$. The discharge frequency increased appreciably when the bathing solution was replaced by one equilibrated

with 20 % $O_2$. Further reductions in the $O_2$ tension of the saline did not necessarily produce a higher rate of discharge. In many instances bathing of the glomus with saline equilibrated with 10 % $O_2$ significantly decreased the discharge if the previous solution was equilibrated with 20 % $O_2$. Solutions equilibrated with 5 % $O_2$ were applied in a few instances, but these low $O_2$ tensions definitely depressed the receptors and left them unresponsive in an irreversible manner.

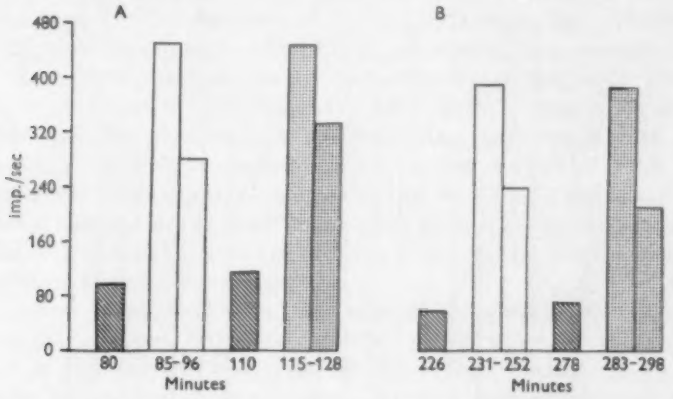


Fig. 2. Effect of different oxygen tensions on carotid-body chemoreceptor discharges. Bars represent average frequency. A, carotid body immersed in saline at 36·4° C flowing at 6·0 ml./min. B, taken from another experiment; glomus in saline at 37·0° C flowing at 4·8 ml./min. Numbers under bars indicate time after excision of the organ. Ordinate, frequency of impulse. ▨ 50 % $O_2$ in $N_2$; □ 20 % $O_2$ in $N_2$; ▤ 10 % $O_2$ in $N_2$.

Figure 2A and B shows that equilibrium of the bathing saline with 20 % $O_2$ may be enough to produce maximal stimulation of the chemoreceptors; A and B represent samples taken from different experiments. In both cases 20 % $O_2$ or 10 % $O_2$ were applied after previous exposure of the organ to 50 % $O_2$ in $N_2$; in every case the vertical bars represent the average frequency of discharge at a given time. The fact that chemoreceptors reached 'saturation' when bathed in saline equilibrated with 20 % $O_2$ may be explained by the fact that the organ was placed in a bath and the dissolved gases had to penetrate into the interior of the organ. This situation may have led to an oxygen gradient within the organ, with the more centrally-located tissues receiving considerably less oxygen than that dissolved in the surrounding saline. However, under certain circumstances maximal chemoreceptor response to inhalation of 20 % $O_2$ also occurred in the animal (Eyzaguirre & Lewin, 1961).

The experiments illustrated in Fig. 2 also show another characteristic of chemoreceptor activity during hypoxia, a decline in impulse frequency occurring shortly after a frequency peak is reached. This decline was clearly and frequently seen in excised preparations, although it was less commonly observed in the animal (cf. Eyzaguirre & Lewin, 1961). The presence of a peak and a later steady state was not due to deterioration of the preparation, since no relationship existed between the presence or magnitude of the peak–steady-state difference with time after excision of the organ. The

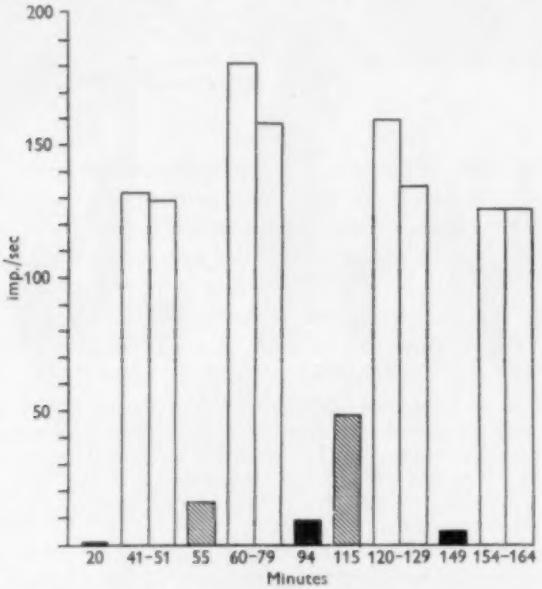


Fig. 3. Same as Fig. 2, but taken from different experiment. Glomus excised from heparinized animal. Saline at 37·0° C flowing at 4·3 ml./min. Numbers under bars indicate time after excision of the organ. Ordinate, frequency of impulses. ■ 100 % $O_2$; other symbols as in Fig. 2.

presence of a frequency peak and later decline was observed in fresh preparations which discharged at very low frequency during administration of pure oxygen. Furthermore, during long experimentation the peak–steady-state ratio usually decreased as time proceeded.

Figure 3 illustrates an experiment in which bathing solutions equilibrated with different concentrations of oxygen were tested on glomerular chemoreceptors early after excision. When the bathing solution was equilibrated with 100 % $O_2$ the nerve discharged at 1/sec. Replacing the solution by another equilibrated with 10 % $O_2$ produced an increase in chemoreceptor

discharge to a peak of 132·7/sec; frequency declined slightly to 129·0/sec in a few minutes and remained stable at this level. The solution was replaced by another equilibrated with 50 % $O_2$ and chemoreceptor frequency declined to 16·2/sec. After several minutes 10 % $O_2$ was again given and this time the discharge rose to 180·8/sec, to decline to a steady level of 157·9/sec. Several minutes later 100 % $O_2$ was administered, followed by application of 50 % $O_2$, and discharges increased from 8·4/sec to 48·0/sec. In this case, once the peak was reached discharges remained at that level for the duration of 50 % $O_2$ administration. Afterwards 10 % $O_2$, pure oxygen and again 10 % $O_2$, were given in succession.

In the experiment just described chemoreceptor frequency rose to higher levels when 10 % $O_2$ was given after 50 % $O_2$ than when it was administered after pure oxygen. Similarly, the peak:steady-state ratio was higher (1·14–1·18) in the first case than in the second (1·0–1·03). This finding would indicate a potentiating effect of the 50 % $O_2$ gas mixture on the initial response of the receptors to lower oxygen mixtures (10 % $O_2$ in this case). This finding, however, was not a general one, since sometimes potentiating effects were not seen.

In general, it seems that the discharge of chemoreceptors to a given oxygen mixture may be either enhanced or depressed by the oxygen tension of the previously applied solution. Consequently, in a given situation the net result produced by hypoxic stimulation may be a delicate balance of these factors. The presence of a frequency decline after an initial frequency peak may be due to 'adaptation' of these receptors. 'Adaptation' may be determined by exhaustion of the chemoreceptors during strong stimulation, since this decline in frequency occurs only when the chemoreceptors are stimulated by low-oxygen mixtures (20–10 % $O_2$). In this connexion it is interesting to notice that stimulation of the olfactory epithelium produces a slow potential which has a peak amplitude followed by a lower amplitude steady state. The peak:steady-state ratio increases during very intense stimulation because the steady state occurs at lower amplitude and this effect has been ascribed to fatigue of the olfactory receptors (Ottoson, 1956).

### *The time course of chemoreceptor frequency changes during exposure of the carotid body to different oxygen tensions*

Chemoreceptors *in vivo* react slowly to hypoxia induced by inhalation of gas mixtures poor in oxygen; their discharge increases to a peak in several minutes. Similarly, when the inhaled oxygen is increased discharges decrease slowly (Eyzaguirre & Lewin, 1961). In those experiments it remained uncertain whether or not part of the slowness of the response was due to a relatively slow lung wash-out. In order to analyse further

the time required for chemoreceptor discharge to reach a new frequency level once the oxygen tension is changed, experiments were designed to measure *in vitro* the time course of chemoreceptor frequency changes obtained during exposure of the glomus to different oxygen mixtures.

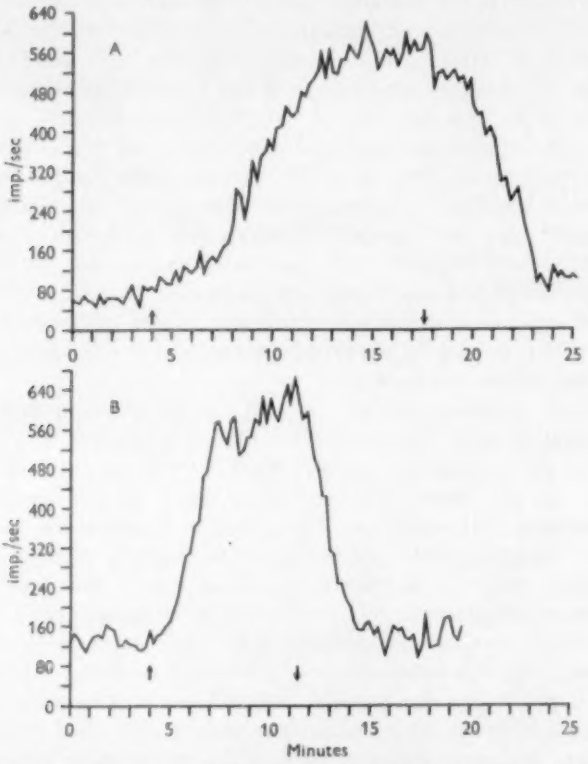


Fig. 4. Effect on chemoreceptor discharge produced by interruption of flow and by lifting preparation into oil. A, glomus immersed in saline equilibrated with 100 % $O_2$ at 35·5° C. At upward arrow, flow (4·2 ml./min) interrupted; at downward arrow flow resumed. B, glomus in saline equilibrated with 100 % $O_2$ flowing at 5·0 ml./min at 36·2° C. At upward arrow, glomus lifted into oil; at downward arrow glomus lowered again into saline.

Such an experiment is illustrated in Fig. 4. Figure 4A shows the results obtained by stopping the flow of saline equilibrated with 100 % $O_2$. Saline flowed at 4·2 ml./min and the discharge frequency averaged 63·4/sec. At the upward arrow the flow was interrupted and the glomus remained in saline. Discharges rose to a peak of 565·3/sec in about 9·5 min. At the downward arrow the flow was resumed at the previous rate and discharges

reached nearly base-line values in about 5·5 min. Figure 4B illustrates another run in the same preparation. The glomus was bathed in saline equilibrated with 100% $O_2$ and the discharge frequency was 131·6/sec. At the upward arrow the glomus was lifted into the paraffin oil covering the saline, while the latter continued to flow; discharges increased to 630·8/sec and reached 70% of this value in 2·8 min. Interruption of the flow probably produces a relatively rapid depletion of the oxygen supply around the glomerular tissues and accumulation of metabolites, factors which are likely to stimulate the chemoreceptors. It is difficult to explain why lifting the organ into oil produces a faster frequency change than that obtained during interruption of the flow. It is possible that oxygen diffuses through oil quite slowly (in spite of the high solubility coefficient), in which case oxygen depletion and accumulation of metabolites would be exaggerated, since the oil is in close contact with the glomus.

Figure 5 illustrates another experiment in which the bathing solution equilibrated with 50% $O_2$ was replaced by another equilibrated with 10% $O_2$. The basal discharge frequency was about 110/sec and rose to 460/sec in about 6 min after changing the solution (upward arrow). Discharges, however, did not stay at this high level of firing and declined to about 325/sec in about 10 min, when they became stable. The dotted line in the illustration shows the time required for the saline in the bath (equilibrated with 50% $O_2$) to be exchanged for that equilibrated with 10% $O_2$. It may be seen that 90% of the exchange occurred in about 2·5 min. The saline-exchange and receptor-frequency curves followed a parallel course with a time lag of about 3·5 min. Figure 5B shows a similar experiment performed in another preparation bathed in a solution equilibrated with 100% $O_2$. The basal discharge averaged 128·4/sec. At the first upward arrow the bathing solution was replaced by another equilibrated with 20% $O_2$; discharges increased to a peak of 627·1/sec in about 6 min. Five minutes after the peak was reached the preparation was again bathed with saline equilibrated with 100% $O_2$ (downward arrow). Discharges came back to base-line levels in about 6 min. The dotted line in this illustration also shows the time course of saline exchange in the system; the receptor-frequency changes lagged about 2·5 min behind the saline-exchange curve.

From the experiments illustrated in Figs. 4 and 5 it is clear that *in vitro* the carotid body chemoreceptors react slowly to changes in the oxygen concentration of the bathing solution. The cause of this slowness has been difficult to determine. One apparent factor can be readily eliminated, i.e. slow mixing of the solutions in the bath. The dotted lines in Fig. 5 indicate that mixing occurs quite early in the dead space of the tube and most of the bathing solution is exchanged a few minutes before the fully

developed oxygen effect is detected. It is possible that two factors may play their parts in slowing the chemoreceptor response to changes in oxygen tensions: (i) slow diffusion of oxygen through the glomerular tissues and (ii) a genuinely slow chemoreceptor activation.

A slow diffusion of oxygen through the glomerular capsule and tissues is possible, although the rate of diffusion through these structures is

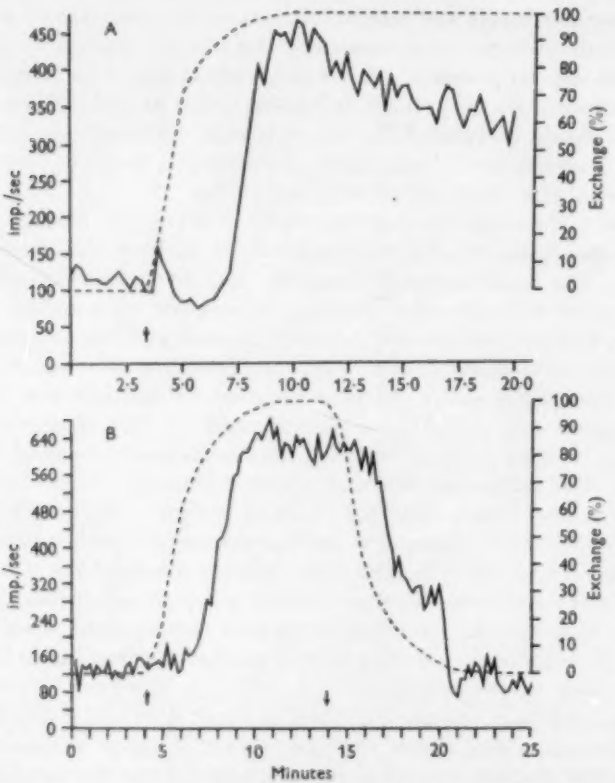


Fig. 5. Time course of chemoreceptor frequency changes produced by reduction of the $O_2$ content in saline. A, glomus immersed in saline equilibrated with 50 % $O_2$ at 36·3° C flowing at 5·8 ml./min. At upward arrow, solution replaced by another equilibrated with 10 % $O_2$. Solid line represents chemoreceptor frequency changes; interrupted line shows time course of solution exchange in the bath. B, from different experiment. Glomus immersed in saline equilibrated with 100 % $O_2$ at 36·2° C and flowing at 4·2 ml./min. At upward arrow solution replaced by another equilibrated with 20 % $O_2$. Solid line, chemoreceptor discharge; interrupted line, time course of solution exchange. Left-hand ordinate, imp./sec; right-hand ordinate, % of saline exchange.

unknown. This point may be resolved by the use of an intraglomerular oxygen electrode.

Without elimination of possibility (i) it is difficult to know whether or not chemoreceptors genuinely react slowly to changes in $O_2$ tension. However, some information is available. Eyzaguirre & Lewin (1961) found that occlusion of the carotid artery produces a near-maximal increase in chemoreceptor discharges in about 25 sec. Also, intracarotid injections of KCN produce a maximal chemoreceptor-frequency change within 6 sec (Alvarez-Buylla, 1951). It is quite clear that the time course of events just described is considerably faster than the time course of frequency changes obtained from the carotid nerve during inhalation of different oxygen mixtures or during exposure of the glomus to different oxygen tensions *in vitro*. How comparable these two types of experiments are is open to question. It is possible, however, that carotid-body chemoreceptors may react maximally to changes in $O_2$ tensions within seconds; the longer periods obtained in this study may be partly due to a slow diffusion of the gas through the glomerular tissues.

*Effect of temperature on chemoreceptor discharge*

An effect of temperature on chemoreceptor discharge may be expected, since glomerular tissues utilize oxygen at a rapid rate (0·00018 ml. $O_2$/carotid body/min, according to Daly, Lambertsen & Schweitzer, 1954). Consequently an increase in temperature should produce a high discharge frequency while a lowering of temperature should produce the opposite effect (see Nashat & Neil, 1955).

Figure 6 illustrates an experiment in which the carotid-body chemoreceptors were bathed with a solution equilibrated with 50 % $O_2$; receptors discharged at about 13/sec at a temperature of 32° C. The temperature of the bath was increased to 38·7 °C in about 7·5 min. The chemoreceptor discharge increased to 280/sec in about 9 min. Discharges stayed at this high level of firing for about 2 min, to decrease to 112/sec in 7–8 min, and remain stable at that level.

The experiment just described shows that chemoreceptors are thermosensitive and that an increase in temperature of the bathing solution increases the frequency of their discharge. The illustration also shows that a rise in saline temperature is followed by an increased discharge after some delay. This delayed response may be accounted for either by the time required to heat the carotid-body receptors and/or by a genuinely slow chemoreceptor activation. This problem may be solved in future experiments by the use of intraglomerular thermistors. The decline in frequency observed in the illustration once the temperature became stable may be

explained by 'adaptation' or exhaustion of these receptors to this form of stimulation.

In general, it was found that temperatures had to be kept at or below 37° C in order to avoid thermal complications during exposure of the carotid body to different $O_2$ mixtures.

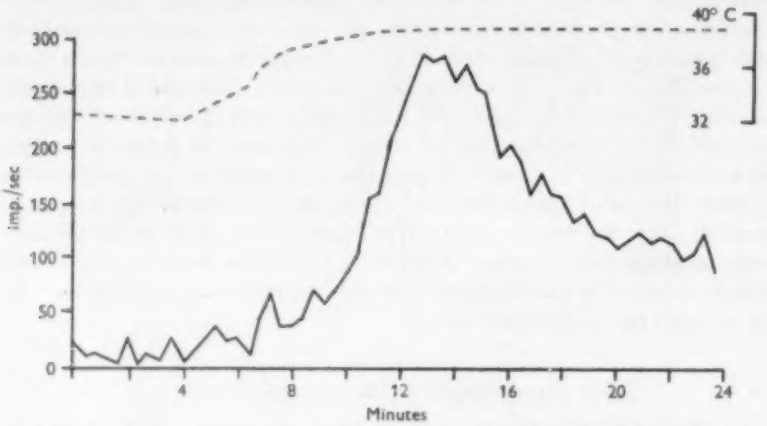


Fig. 6. Effect of temperature on chemoreceptor discharge. Saline equilibrated with 50 % $O_2$ flowing at 5·0 ml./min. Solid line, chemoreceptor discharge frequency; interrupted line, temperature of bathing solution.

## DISCUSSION

Carotid-body chemoreceptors survive for several hours after being excised from the animal and placed in a bath containing a physiological solution. In this respect the carotid body does not differ from other tissues where similar procedures have been employed.

The carotid-body chemoreceptors respond *in vivo* to $O_2$ lack in a manner similar to that observed *in vitro*, although in the later state their response to low oxygen is better preserved. *In vitro* the maximal discharge obtained at different $O_2$ levels tends to become lower as time proceeds, being about 40 % of the initial response 4–5 hr after excision. This decay of the preparation may be due to lack of $CO_2$ in the bathing saline and to inadequate oxygenation of deeper glomerular structures. However, in spite of the limitations of the method it is felt that the good survival of the receptors in a bathing medium provides an excellent preparation for future studies of chemoreceptor function.

A point that must be emphasized is that carotid-body chemoreceptors are perfectly capable of responding to $O_2$ lack in the total absence of haemoglobin. This finding lends support to the idea that, normally, these

receptors are activated by a decreased oxygen tension of the plasma rather than by a reduction in the oxygen saturation of haemoglobin (Comroe & Schmidt, 1938; Duke *et al.* 1952). However, the *in vitro* experiments do not exclude some role of Hb in the activation of chemoreceptors; they merely indicate that Hb presence is not essential.


## SUMMARY

1. Chemoreceptors of the cat's carotid body have been studied *in vitro* by placing the organ in flowing Locke's solution equilibrated with gas mixtures containing different amounts of $O_2$. Chemoreceptor discharges have been recorded from the carotid nerve.

2. Chemoreceptor discharge frequency is very low if the carotid body is bathed with saline equilibrated with 100 % $O_2$. They increase if the bathing solution is replaced by another equilibrated with 50 % $O_2$. Further reductions in the $O_2$ tension of the saline brings in more discharges, but a maximum is attained by bathing the organ with solutions equilibrated with 20 % $O_2$ or 10 % $O_2$.

3. If discharges have reached a higher level after reducing the bathing $O_2$ from 100 to 50 % $O_2$ they stay at that level. If, however, $O_2$ is reduced from 100–50 % $O_2$ to 20–10 % $O_2$ a higher peak is reached but the discharge frequency declines to a lower but stable level shortly afterwards. This probably constitutes an adaptation phenomenon.

4. When the $O_2$ of the bathing solution is reduced, the chemoreceptor discharge reaches a new peak in several minutes. This delay may be due to slow penetration of $O_2$ into the glomerular tissues and/or to a genuinely slow chemoreceptor activation.

5. Increasing the temperature of the bathing fluid above 37° C produces a marked increase in chemoreceptor discharge. Discharges, however, decline to a lower level a few minutes after reaching the peak.


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# THE EFFECT OF SYMPATHETIC STIMULATION ON CAROTID NERVE ACTIVITY

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An effect of sympathetic activity on carotid-body chemoreceptor discharge might be expected, since the carotid body receives an abundant nerve supply from the superior cervical ganglion (de Castro, 1926). Moreover, Floyd & Neil (1952) have found a small but definite increase in carotid-nerve activity when the sympathetic branches supplying the carotid body were stimulated. Hereafter, the nerve(s) linking the superior cervical ganglion with the carotid body will be referred to as the ganglio-glomerular nerve(s). This term is not entirely adequate, since many of the nerve fibres join the carotid nerve close to the glomus; they continue in such a nerve to join the glossopharyngeal nerve (see Eyzaguirre & Uchizono, 1961). Floyd & Neil interpreted this effect as increased chemoreceptor discharge. A significant problem remains, however, namely whether or not sympathetic effects, if present, are due to a direct action upon the sensory receptors or to construction of local vessels. In other sensory receptors such as tactile units of the frog's skin (Loewenstein, 1956) and mammalian spindles (Hunt, 1960) sympathetic actions may be exerted by liberation of adrenaline or noradrenaline from the sympathetic terminals, acting directly on neighbouring nerve endings (for an opposite view see Eldred, Schnitzlein & Buchwald, 1960). In the case of the carotid-body chemoreceptors sympathetic effects which have been observed might well be indirect, since such studies have been performed in the whole animal where a reduction of glomerular blood flow is known to occur during stimulation of the cervical sympathetic trunk (Daly, Lambertsen & Schweitzer, 1954).

In the present study the possibility of a direct sympathetic action on chemoreceptors was investigated *in vitro*, where vascular effects are absent, and the results obtained in the excised preparation were compared with similar observations in the whole animal. Results presented below indicate that stimulation of fibres emerging from the superior cervical ganglion

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*in vivo* produces only a small increase in chemoreceptor discharge apparently due to vasoconstriction. No evidence of a direct sympathetic effect either on the sensory nerve endings or on glomerular cells has been found. However, a number of non-myelinated (C) fibres of sympathetic origin elicit potentials in the carotid nerve upon stimulation and their response may be confused with normally occurring sensory discharges. A preliminary report has already been published (Eyzaguirre, Uchizono & Lewin, 1961).

## METHODS

Adult cats were anaesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories) 40 mg/kg I.P. One series of experiments was performed in the animal while another series was done *in vitro*. For experiments performed *in vivo* the animal was allowed to breathe the room air. Its rectal temperature was kept at 37–38° C. The neck was opened in order to expose the superior cervical ganglion, its preganglionic fibres and its glomerular connexions. The exposed surface was covered with warm mineral oil. Stimulating electrodes were placed either on the preganglionic trunk or on the ganglio-glomerular nerve. Nerve action potentials recorded from the carotid nerve were fed to an amplifier, which in turn was connected to a double-beam oscilloscope and to a counter-printer system. With this system the potentials could be displayed, photographed and their frequency per second recorded once every 2 sec (cf. Eyzaguirre & Lewin, 1961*a*). In some animals recording of carotid nerve impulses was accomplished 2–6 days after aseptic glossopharyngeal section in the neck performed under sodium pentobarbital anaesthesia. For *in vitro* experiments the carotid body and its own nerve plus a stretch of carotid artery were excised together with their sympathetic connexions, i.e. the superior cervical ganglion, a length of the preganglionic trunk and the ganglio-glomerular nerve. The excised tissues were placed in a Petri dish containing Locke's solution for further dissection and removal of unwanted connective tissues. Care was taken to decapsulate the ganglion soon after excision in order to facilitate diffusion of substances. The tissues were then mounted in a transparent chamber containing Locke's solution (pH 7·4) at about 36° C, previously mixed with glucose 1·0 g/l. The saline was bubbled with a mixture of 94 % $O_2$ and 6 % $CO_2$ and covered by a layer of mineral oil. When substances were applied locally the whole preparation was placed in oil and the desired portion was lowered into a small box containing the substance dissolved in Locke's solution (cf. Eyzaguirre, 1960). Stimulating electrodes could be placed either on the sympathetic preganglionic fibres, on the ganglio-glomerular nerve or on the carotid nerve. Recording of action potentials from the carotid or ganglio-glomerular nerves was accomplished in a manner similar to that already described for experiments *in vivo* (see also Eyzaguirre & Lewin, 1961*b*).

The membrane potential of glomerular cells was measured with intracellular micropipettes filled with 3 M-KCl, having an a.c. resistance of from 10·5 to 70 MΩ. The glomerular capsule proved too tough for easy penetration of the micro-electrode, consequently the glomus was immersed in a solution of chymotrypsin (1 mg/ml.) in Locke's solution and digestion was allowed to proceed for 30–60 min. After this procedure the sensory discharges did not change and penetration of the glomerular cells was readily accomplished. Since membrane potentials were low the tip potential of each electrode was measured (cf. del Castillo & Katz, 1955). Tip potentials ranged from less than 5 to 45 mV but they did not appreciably alter the recorded membrane potential values.

## RESULTS

### *Changes in carotid-nerve impulses during sympathetic stimulation*

*Stimulation of preganglionic fibres.* In the cat, repetitive preganglionic stimulation at 5–10/sec produced an increase in the frequency of carotid-nerve impulses. Once stimulation was discontinued the frequency declined rapidly to base-line levels (Fig. 1A). In order to determine whether or not this effect was acting upon chemo- or pressoreceptor, the carotid nerve was split into a number of small filaments; care was taken to record activity from chemoreceptors to the exclusion of pressoreceptor discharges. Such an experiment is presented in Fig. 1B, where it may be seen that sympathetic effects on this small filament are quite similar to those obtained from the whole nerve. Splitting the nerve revealed that preganglionic stimulation produced, besides the normally occurring sensory discharges, a polyphasic potential which appeared after a constant latency (Fig. 2A). The waves of this complex were small enough in the intact nerve to be interspersed with the background discharge, thus adding to the counts of sensory impulses.

The contribution of sympathetic fibres to carotid-nerve activity was further analysed by recording from carotid nerves in which afferent fibres had been caused to degenerate by section of the nerve centrally from 2 to 6 days before the experiment. These nerves showed little or no background discharge. Sympathetic stimulation evoked a compound action potential in the carotid nerve, which appeared after a constant latency. These potentials were clearly recognized and photographed from the oscilloscope screen (Fig. 2B and C); they corresponded to activity derived from a fairly large number of C fibres (see later). The C-fibre potentials thus evoked were relatively rapid and were readily recorded by the electronic counter, as shown in Fig. 1C. At rest the counter picked up some of the amplifier noise. Between arrows the sympathetic trunk was stimulated at 10/sec and the sudden appearance of the sympathetic potentials was evident by the rapid increase in the counts. These potentials disappeared abruptly once the stimulator was turned off. Experiments performed *in vitro* yielded essentially similar results, although the potentials of sympathetic origin were more conspicuous, owing to less background discharge (see below).

In conclusion it may be stated that the appreciable increase in counts obtained from the carotid nerve during preganglionic sympathetic stimulation is largely produced by the appearance of polyphasic potentials of sympathetic origin. This would also appear in records obtained from the oscilloscope screen and it may lead one to assume, erroneously, that chemoreceptor discharges have increased.

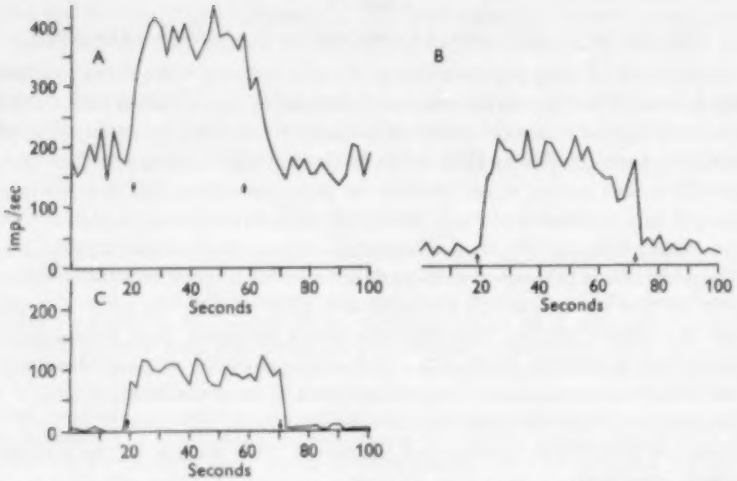


Fig. 1. Changes in carotid nerve impulses during sympathetic stimulation. Preparation *in situ*. A, B and C, stimulation of preganglionic sympathetic trunk at 10/sec. A, recording from the whole nerve; B, pressoreceptors eliminated by dissection; C, recording from carotid nerve centrally severed 6 days previously.

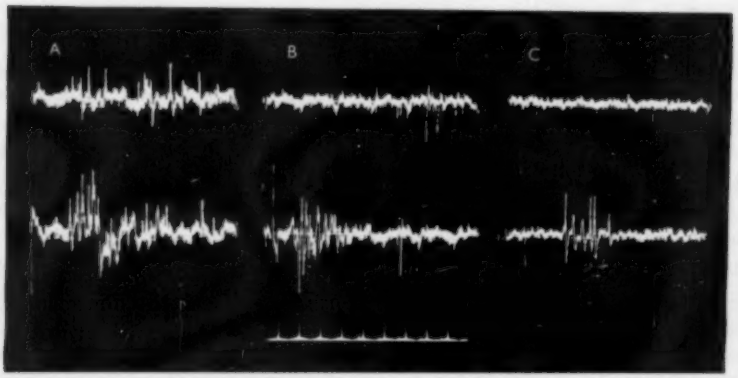


Fig. 2. Effect of single pre-ganglionic shocks on carotid nerve impulses. Preparation *in situ*. In all columns the upper traces represent base-line discharge; lower traces show the evoked sympathetic potentials. A, recording from normal carotid nerve; B, carotid nerve centrally severed 48 hr previously; C, recording 6 days after central section of carotid nerve. Time marker, 10 msec.

*Stimulation of post-ganglionic fibres.* Since preganglionic sympathetic stimulation increased the counts from the carotid nerve, sometimes showing a short period of facilitation and a gradual return to resting discharge frequency, it was suspected that a small sympathetic effect on chemoreceptor discharge might be present. In order to study this effect a series of experiments was performed in the animal and another series *in vitro*. In both cases the superior cervical ganglion was removed and the ganglio-glomerular nerve was placed on stimulating electrodes. Removal of the ganglion was carried out in order to avoid ganglionic facilitation during repetitive stimulation. Recording was done in the animal from small carotid-nerve filaments containing no pressoreceptor fibres; *in vitro* recording was done from the whole nerve but pressoreceptor discharges were eliminated by keeping the artery unstretched and by removing the adventitia (cf. Alvarez-Buylla, 1954; Eyzaguirre & Lewin, 1961*b*).

Stimulation of the ganglio-glomerular nerve *in vivo* usually did not produce any change in the frequency of chemoreceptor discharge. In a few instances, however, a small but definite increase was obtained. In these experiments the relatively slow potential recorded from the carotid nerve and evoked by ganglio-glomerular stimulation was not recorded by the counter. The instrument was gated in such a way as to avoid relatively slow waves. Such an experiment is illustrated in Fig. 3A. Between the arrows the ganglio-glomerular nerve was stimulated at 10/sec and a slow increase in chemoreceptor frequency was obtained.

The small increase in chemoreceptor activity produced by sympathetic stimulation observed in the cat could be explained either by a direct sympathetic action on these receptors or by glomerular vasoconstriction (Daly *et al.* 1954). These possibilities were elucidated by studying the effect of sympathetic stimulation on carotid-nerve activity in the excised preparation. Such an experiment is illustrated in Fig. 3B. The relatively low background discharge may be readily explained, since the preparation was bathed with saline equilibrated with 94 % $O_2$ and 6 % $CO_2$. Between the arrows the ganglio-glomerular nerve was stimulated at 10/sec. No changes in chemoreceptor activity could be detected. In all cases studied *in vitro* chemoreceptor frequency remained unchanged during sympathetic stimulation. It is concluded that the small increase in chemoreceptor discharge obtained in the animal is due to glomerular vasoconstriction, since direct sympathetic effects on chemoreceptor discharges have not been found.

### *The complex potential evoked in the carotid nerve by preganglionic sympathetic stimulation*

The experiments already described showed the presence of a polyphasic potential complex in the carotid nerve during sympathetic stimulation

(Fig. 2). However, owing to the abundant background discharge, such potentials were clearly recorded in the animal only after elimination of a number of afferent fibres by dissection or by degeneration. An analysis of the sympathetic potentials obtained from the whole carotid nerve was possible in excised preparations when the artery was unstretched, the adventitia removed and the preparation bathed in saline equilibrated with a high concentration of oxygen. With these procedures the sensory discharge background was drastically reduced and appropriate placing of the recording leads brought the sympathetic potentials into full view.

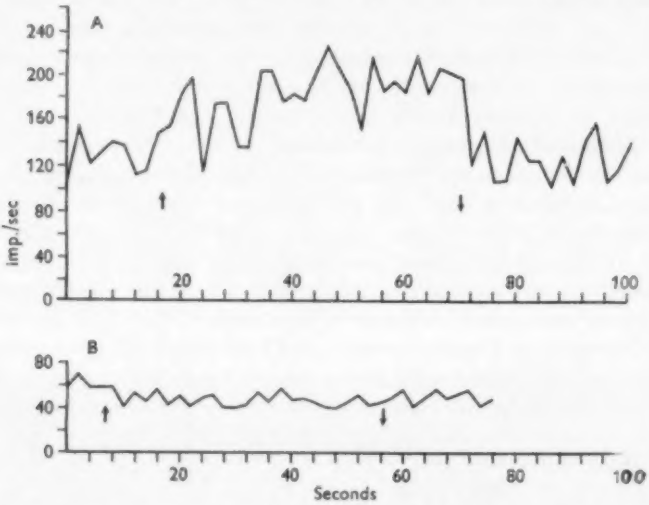


Fig. 3. Increase of chemoreceptor discharge during ganglio-glomerular stimulation in the animal and lack of effect *in vitro*. A, recording from carotid nerve *in situ*. B, recording from carotid nerve *in vitro*. In A and B ganglio-glomerular nerve stimulated at 10/sec.

The carotid-nerve potential of sympathetic origin was studied during stimulation of the preganglionic fibres with single and double shocks at varying intervals. Potentials from the carotid nerve and from the sympathetic ganglion were simultaneously recorded by independent leads which were connected to the double-beam oscilloscope through separate amplifiers. The ganglionic potentials were registered by placing the leads on the ganglion, one at each pole. Figure 4 illustrates an experiment in which single shocks of increasing intensity were delivered at 0·6/sec. In the illustration the upper trace shows the potentials recorded from the carotid nerve, while the lower trace shows the potentials recorded from the sympathetic ganglion. Submaximal preganglionic volleys produced a

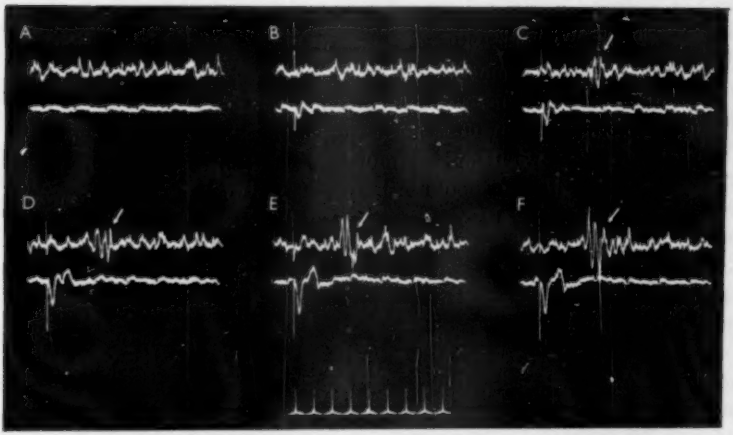


Fig. 4. Effects of preganglionic shocks of increasing intensity on carotid-nerve impulses. The upper traces show recording from carotid nerve while the lower ones show potentials obtained from the superior cervical ganglion. Excised preparation. A, base lines; B, C, D, E, and F, potentials obtained by increasing stimulus strength. Arrows indicate the carotid nerve potential of sympathetic origin. Time marker, 10 msec.

Fig. 5. Same as Fig. 4 but this time double shocks at varying intervals were applied to the preganglionic sympathetic fibres. Note the dispersion and disappearance of the second carotid nerve potential at close stimulating intervals. Time marker, 10 msec.

small polyphasic action potential in the carotid nerve, which increased in amplitude as the stimulus strength was increased. The total duration of this complex was about 10 msec (arrows). This temporal dispersion indicates that maximal preganglionic volleys activate fibres of different conduction velocities. The intervals between the different peaks of the polyphasic complex were too long (more than 2 msec) to be accounted for only by synaptic delay. When in the same experiment two shocks were given at decreasing intervals, the second carotid-nerve potential became smaller and temporal dispersion even more noticeable (Fig. 5). Figure 5B and C shows that at shock intervals of 50 and 45 msec respectively the polyphasic response of the carotid nerve (upper beam) is relatively well preserved after the second shock, but there is a deficit in the amplitude of the second ganglion potential (lower trace). When the second shock was given at intervals of 18 msec a smaller and highly polyphasic potential of longer duration was recorded from the carotid nerve following a smaller ganglion potential. When the shocks were separated by an interval of 10 msec the second carotid-nerve potential was even smaller and more dispersed (Fig. 5G). At shorter intervals (8 and 6 msec respectively) the second carotid-nerve complex became even more dispersed and was hardly noticeable (Fig. 5H and I).

*Neuronal origin of the sympathetic fibres in the carotid nerve*

Experiments were designed to study whether or not the sympathetic fibres in the carotid nerve had their synaptic origin in the superior cervical ganglion. In the excised preparation the tissues were lifted into oil and the sympathetic ganglion was lowered into a chamber containing a solution of a ganglionic blocking agent. The sympathetic preganglionic fibres were stimulated by maximal shocks at 0·6/sec. One pair of recording electrodes registered activity from the carotid nerve while another pair of leads was placed on the ganglio-glomerular nerve.

Metubine (dimethyl-tubocurarine iodide; Lilly) in concentrations of $5 \times 10^{-6}$ to $5 \times 10^{-4}$ (w/v) or hexamethonium (hexamethylenebis(trimethylammonium chloride); Burroughs Wellcome) in concentrations of $5 \times 10^{-6}$ (w/v) were employed. The results obtained with either substance were similar: the sympathetic ganglion was blocked and the complex potential evoked in the carotid nerve by preganglionic sympathetic stimulation disappeared. Such an experiment is illustrated in Fig. 6. Figure 6A shows the base-line discharge. In figure 6B the upper trace illustrates the evoked carotid-nerve action potential complex lasting about 12 msec. The lower trace shows the potential registered from the ganglio-glomerular nerve. Between B and C, hexamethonium was applied to the ganglion. In D, 7 min after application of the drug, the carotid-nerve

complex is reduced in amplitude and more dispersed. The ganglio-glomerular nerve potential is also smaller. These phenomena continue their course in E and F. In G the carotid-nerve complex has disappeared almost entirely while very little is left of the ganglio-glomerular potential. After complete ganglionic block the stimulating electrodes were placed on the ganglio-glomerular nerve and single shocks evoked a large potential in the carotid nerve (not illustrated).

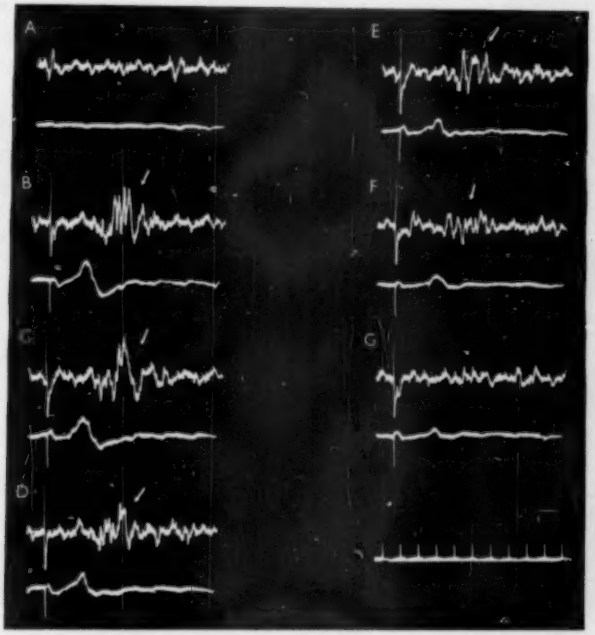


Fig. 6. Effect of hexamethonium applied to the superior cervical ganglion on the carotid-nerve potential of sympathetic origin. Upper traces show the carotid-nerve impulses while the lower traces show the potentials obtained from the ganglio-glomerular nerve near the ganglion. Excised preparation. A, base line; B, control record showing the effect of a single shock applied to preganglionic sympathetic fibres; C, 3 min after local application of hexamethonium ($5 \times 10^{-6}$ (w/v)); D, 7 min after the drug; E, 11 min after $C_6$; F, 14 min after the drug; G, 34 min after $C_6$. Time marker, 10 msec.

Essentially similar results were obtained in the animal when recording was done from filaments of a normal nerve or one in which the afferent fibres had degenerated. Hexamethonium in doses of 5 mg/kg given intravenously completely abolished the carotid-nerve sympathetic potential in a few seconds. After several minutes the potential returned to normal.

From these experiments it is concluded that most of the sympathetic fibres giving rise to activity in the carotid nerve had their origin in the superior cervical ganglion.

*Absence of synapses in the carotid-nerve sympathetic pathway at the glomerular level*

The preceding section showed that the majority of the carotid-nerve sympathetic fibres must have their somata in the superior cervical ganglion. However, the possibility exists that some synapses with ganglion cells could have been missed. Consequently, experiments were designed to test

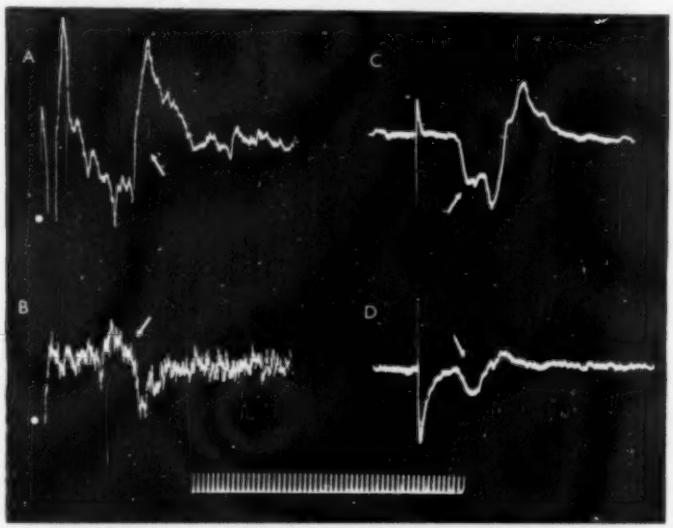


Fig. 7. Recording of C-fibre potentials from carotid and ganglio-glomerular nerves. Excised preparation. A and C show the sympathetic potential recorded from the carotid nerve during stimulation of the ganglio-glomerular nerve. B and D show the sympathetic potentials recorded from the ganglio-glomerular nerve during stimulation of the carotid nerve. Note the coincidence of peaks as shown by arrows. Time marker, 1 msec.

for the presence of synapses in the carotid-nerve sympathetic pathway in or around the glomus. Experiments were performed to search for a synaptic barrier to 'antidromic' stimulation and cholinergic blocking agents were applied to the glomus.

*'Antidromic' stimulation.* In a set of experiments the excised preparation was left in oil while stimulating electrodes were placed on the ganglio-glomerular nerve and potentials recorded from the carotid nerve. After a characteristic potential complex was obtained the stimulating and recording

leads were reversed, care being taken to avoid displacement of the electrodes. In both instances the cathode was nearer to the first recording electrode. Such an instance is exemplified in Fig. 7. Figure 7A illustrates the potentials obtained by single maximal shocks delivered to the ganglio-glomerular nerve while potentials were recorded from the carotid nerve; B shows the reverse situation. It is evident in this column that the first complex (dot) elicited by 'orthodromic' stimulation is not properly recorded during 'antidromic' stimulation because it rides partly on the artifact. But the second 'orthodromic' complex (arrows) is clearly present in the 'antidromic' situation and their peaks coincide exactly. In both cases, however, the 'antidromic' potentials are considerably smaller, owing to the less favourable recording conditions. Indeed, the ganglio-glomerular nerve is short and thick, possessing a strong sheath which contains many fibres that do not travel in the carotid nerve (Eyzaguirre & Uchizono, 1961). Figure 7C, obtained from a different preparation, shows the 'orthodromic' potential presenting two peaks which correspond to the slowest components already described (potentials marked by arrows). In this experiment, the earlier wave, shown in the left-hand column (dot) was not obtained. Figure D shows the 'antidromic' potential (arrow) whose peak corresponds to the first peak of the deflexion obtained in C. The second peak of C did not appear clearly in D although it was visible when high amplification was used.

The smaller size of the potential recorded from the ganglio-glomerular nerve as compared to that obtained from the carotid nerve could be explained because of unfavourable recording conditions. However, the possibility still remained that a small number of synapses could have been missed in the 'antidromic' situation. Consequently, a second series of experiments was performed as shown below.

*Application of cholinergic blocking agents.* The excised tissues were lifted into oil while the glomus was lowered into a small chamber containing a solution of a cholinergic blocking substance. Care was taken to eliminate by dissection most of the connective tissues surrounding the carotid body to improve penetration of substances. Hexamethonium ($5 \times 10^{-5}$ (w/v)), Decamethonium (Decamethylenebis(trimethylammonium bromide); Burroughs Wellcome) in doses of $2{\cdot}5 \times 10^{-6}$ (w/v) or atropine sulphate ($10^{-5}$ (w/v)) were applied in different experiments. Results were on the whole negative, since none of these substances appreciably changed the carotid nerve potential evoked by ganglio-glomerular stimulation. Essentially similar results were obtained in the animal when these substances were injected intravenously while the ganglio-glomerular nerve was stimulated and potentials were recorded from the carotid nerve. These drugs would be expected to block any type of cholinergic synapses if present.

From these experiments it is concluded that most of the sympathetic fibres giving rise to activity in the carotid nerve had their origin in the superior cervical ganglion.

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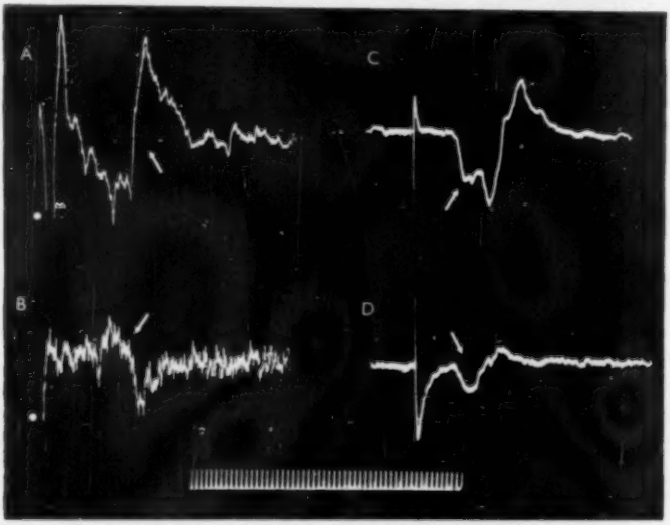


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Results presented in this section show that cholinergic synapses do not occur in the carotid-nerve sympathetic pathway once the fibres leave the superior cervical ganglion and reach the glossopharyngeal nerve. Some myelinated fibres, however, leave the ganglion to innervate some microganglia located near the glomus. The short post-synaptic fibres from the microganglia innervate some glomerular blood vessels (de Castro, 1926) but they do not seem to continue in the carotid nerve (see also Eyzaguirre & Uchizono, 1961).

*Conduction velocity of the sympathetic fibres in the carotid nerve*

In order to have an estimate of the conduction velocity of the fibres producing the sympathetic component of the carotid-nerve complex, the ganglio-glomerular nerve was stimulated and action potentials recorded from the carotid nerve in the excised preparation. The distance between the stimulating and recording electrodes was kept at 5 mm as measured with a calibrated eyepiece. In general, stimulation of the ganglio-glomerular nerve produced one or two complexes in the carotid nerve, apparently due to activation of two distinct fibre groups. One group had a conduction velocity of about 1·8 m/sec (potential marked by a dot in Fig. 7), the other 0·3–0·4 m/sec (potentials marked by arrows in Fig. 7). A better view of the potentials elicited by fibres having slower conduction velocity is shown in Fig. 8, where shocks of increasing intensity were delivered. In fig. 8A and B, a potential of increasing amplitude was obtained with a shock–peak latency of 13·2 msec. Stronger shocks brought in a second potential with a latency of 17·8 msec, which started to appear in C and which is quite evident in D, E and F. The conduction velocity of the fibres involved was calculated as 0·4 m/sec for those forming the first complex and 0·3 m/sec for those eliciting the second complex. It must be understood, however, that the conduction velocity estimates are only approximate; the electrodes could not be displaced to take measurements at different points in the nerve, which was too short for such a manoeuvre.

The experiments illustrated in Figs. 7 and 8 show that stimulation of the ganglio-glomerular nerve evokes activity in two distinct fibre groups, which may be recorded from the carotid nerve. Assuming that conduction velocity in such fibres is directly related to diameter by a factor of 1·7 (Gasser, 1955) one may estimate that one group of fibres has a diameter of about 1 $\mu$ while the other group has a diameter of from 0·1 to 0·3 $\mu$.

Further information on the two types of slowly-conducting fibres was obtained by dissecting the carotid nerve into small filaments and recording the response obtained by stimulation of the ganglio-glomerular nerve. In several instances unitary activity of sympathetic fibres was obtained, since

the potentials showed a sharp threshold and all-or-none characteristics. Such an instance is illustrated in Fig. 9. Figure 9A is the base-line discharge from a small carotid-nerve filament. B and C show the small potential (marked by dots) evoked by stimulation of the ganglio-glomerular nerve. The conduction velocity of this unit was 1·66 m/sec. Figure 9D, E and F are from a different experiment. D shows the base-line discharge,

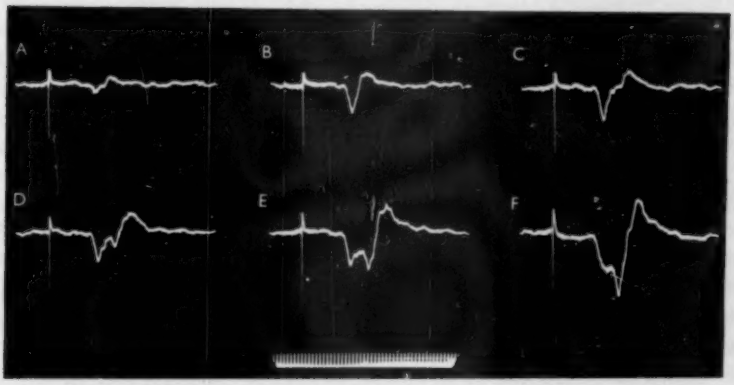


Fig. 8. Stimulation of ganglio-glomerular nerve and recording from carotid nerve. Excised preparation. From A to F, shocks of increasing intensity applied to the nerve. Time marker, 1 msec.

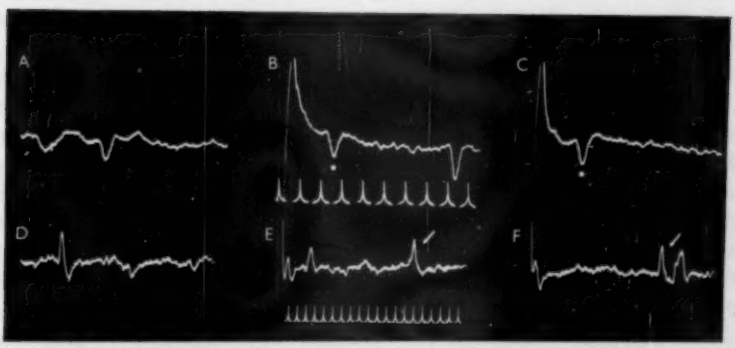


Fig. 9. Recording of single C-fibre potentials from carotid nerve. Excised preparation. Stimulation of ganglio-glomerular nerve at 1/sec. The two rows illustrate results obtained from two different experiments. A, B and C taken from same experiment. A, base line; B and C, a small C-fibre potential is recorded near the artifact (dots). D, E and F taken from different experiment. D, base-line discharge; E and F illustrate a C-fibre potential obtained from carotid nerve (arrows). Close to the artifact (E and F) a potential from myelinated fibre conducting at 5 m/sec is seen. Note similarity of C-fibre potential with normally occurring sensory discharge. Time markers, 1 msec.

while E and F illustrate the effect of single shocks delivered to the ganglio-glomerular nerve. A potential was evoked (arrows) from a unit which conducted at 0·34 m/sec, which clearly falls into the C-fibre group.

The fact that the amplitude of chemoreceptor impulses in Fig. 9 is of the same order of magnitude as that evoked by sympathetic stimulation poses the question whether or not in this case the afferent discharges were conveyed by sensory C fibres. Non-myelinated sensory elements have been described in the rabbit's carotid nerve (Douglas & Ritchie, 1956). C fibres, presumably subserving a sensory function, also occur in the homologous nerve of the cat (Eyzaguirre & Uchizono, 1961).

*Lack of membrane potential changes in glomerular cells during sympathetic stimulation*

De Castro (1926) has indicated that sympathetic fibres from the superior cervical ganglion are distributed in the glomerular interstitial spaces without making contact with the major glomerular cells. However, the possibility exists that stimulation of the sympathetic nerves might produce a detectable change in the membrane potential of the glomerular cells. Consequently, the glomerular cells were impaled with micro-electrodes and the sympathetic nerves were stimulated by placing electrodes on the cervical sympathetic trunk or on the ganglio-glomerular nerve. A total of 450 cells were impaled in this series. They showed low resting potentials (10–50 mV), the mode of the frequency–distribution curve being 20 mV. Sympathetic stimulation did not change the membrane potential of any of the cells studied.

In all probability the impaled cells belonged to the so-called 'chief' cells, since these are the largest and most numerous of glomerular cells (Adams, 1958). Their diameter ranges from 6 to 10$\mu$ (Ross, 1959). The other cellular elements in the carotid body (sustentacular cells, etc.) are probably too small to allow easy penetration of the micro-electrode. The low resting potentials obtained could have been due partly to injury produced by the insertion of the micro-electrode. However, resting potentials appeared suddenly during penetration and were fairly well maintained for periods long enough to test the effect of sympathetic stimulation.

From a physiological point of view these experiments have not decided the question of a synaptic contact between sympathetic fibres and glomerular cells. It is possible that sympathetic action might elicit some form of activity in these cells which does not appear as a resting-potential change.

## DISCUSSION

The primary purpose of this study was to determine whether or not the carotid-body chemoreceptors are provided with an efferent control through fibres originating in the superior cervical ganglion. The observations reported here show that the role played by cervical sympathetic nerves in modulating the chemoreceptor discharge is small. Sympathetic effects when present seem to be entirely vascular. This finding falls into line with the observations of Daly *et al.* (1954), who found sometimes an 87% increase in glomerular vascular resistance when the cephalic end of the cut cervical sympathetic trunk was stimulated. Direct sympathetic effects on the chemoreceptors seem to be absent, since, in the excised preparation stimulation of the cervical sympathetic nerves did not change the frequency of the chemoreceptor discharge or the membrane potential of the 'chief' glomerular cells.

In the search of an efferent control of the chemoreceptors one would have to look into possible efferent contacts provided by fibres of the carotid nerve. In this connexion it is worth noting de Castro's observation (1926, 1951) that degeneration of the ganglio-glomerular nerves does not change appreciably the fine non-myelinated plexus which innervates the arteries and arterioles of the glomus. It is his contention that a great part of the vascular innervation is provided by small non-myelinated fibres originating from a few ganglion cells interspersed among carotid-nerve fibres near the glomus. The preganglionic fibres of these structures have apparently a medullary origin. Whether or not activity of some of these (or other) fibres may modulate the chemoreceptor discharges is unknown.

A positive finding in this study is the presence of a nervous pathway of sympathetic origin in which fibres originating in the superior cervical ganglion course toward the glomus, where numerous fibres continue in the carotid nerve and then join the glossopharyngeal nerve. Some of the fibres, however, leave the glomus to innervate other regions in the neck (Eyzaguirre & Uchizono, 1961). Most of the carotid-nerve sympathetic fibres are non-myelinated, judging from their conduction velocities, and fall into two definite groups. One group conducts at 0·3–0·4 m/sec while the other group conducts at 1·6–1·8 m/sec. Their final destination and function is unknown.

## SUMMARY

1. The effect of sympathetic stimulation on the carotid body chemoreceptors discharge was investigated both in the animal and on the carotid body *in vitro*. In the animal sympathetic stimulation may induce a small increase in chemoreceptor discharge. This effect is due to vasoconstriction,

since chemoreceptor discharges are not influenced by sympathetic activity *in vitro*.

2. Stimulation of preganglionic sympathetic fibres produces a polyphasic potential in the carotid nerve after a constant latency. This potential persists in the nerve even 6 days after severance of the glossopharyngeal nerve. The fibres giving rise to the carotid-nerve sympathetic potential have their somata in the superior cervical ganglion, since hexamethonium (injected into the animal or applied locally) blocks such a response.

3. The fibres producing the carotid-nerve sympathetic potential are mainly non-myelinated (C fibres). Two groups of fibres have been found: one group has a conduction velocity of from 1·6 to 1·8 m/sec while the second group of fibres conduct at 0·3–0·4 m/sec. Their final destination or function is unknown. This sympathetic pathway seems to pass by the glomus without the interposition of cholinergic synapses in or around the carotid body.

4. The membrane potential of the 'chief' glomerular cells (10–50 mV) remains unchanged during sympathetic stimulation.

5. It is concluded that the sympathetic control of the carotid body chemoreceptors is provided only through vascular effects. Other possible efferent pathways are discussed.

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# OBSERVATIONS ON THE FIBRE CONTENT OF NERVES REACHING THE CAROTID BODY OF THE CAT

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The region of the carotid sinus and body is innervated principally by the carotid nerve, a branch of the glossopharyngeal nerve. It also receives nerve branches from the superior cervical ganglion (ganglio-glomerular nerves). The principal afferent innervation reaches the carotid sinus and glomus through the carotid nerve (Adams, 1958). This nerve contains about 650 myelinated fibres, the majority being from presso- or chemoreceptors. Some of the myelinated fibres have been considered, however, to be efferent to a small number of nerve cells located near the termination of the carotid nerve in the glomus (de Castro, 1926, 1951). Physiological studies have indicated the presence of non-myelinated (C) fibres in the carotid nerve as well. Douglas & Ritchie (1956) recorded potentials conducting at C-fibre velocity when stimulating and recording from the carotid nerve. Also, stimulation of the ganglio-glomerular nerves gives rise to an action potential in the carotid nerve which also is propagated at a velocity characteristic of C fibres (Eyzaguirre & Lewin, 1961*b*). Anatomical evidence that the ganglio-glomerular nerve is mainly comprised of non-myelinated fibres originating in the superior cervical ganglion has been presented by de Castro (1926).

The purpose of the present study is to analyse further the innervation of the carotid-body region, since several details have remained uncertain: (1) the course followed by the carotid nerve once it reaches the carotid-body–sinus area to innervate the glomus and the sinus; (2) how the ganglio-glomerular nerve fibres reach the glomus and how they join the carotid nerve; (3) whether or not non-sympathetic C fibres occur in the carotid nerve. Also (4) no anatomical studies have appeared indicating either the presence of C fibres in the carotid nerve or their diameter ranges and distributions; similarly, data on the fibre size and diameter distribution of

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the non-myelinated axons in the ganglio-glomerular nerve are not available. A preliminary report has already been published (Eyzaguirre, Uchizono & Lewin, 1961).

## METHODS

The carotid body and its own nerve in addition to the ganglio-glomerular connexions were excised from cats under sodium pentobarbital, (Nembutal; Abbott Laboratories) 40 mg/kg I.P. For direct observation the tissues were placed in a Petri dish containing Locke's solution and examined with a dissecting microscope under transmitted polarized light. For examination under the phase-contrast and electron microscopes the tissues were excised from the animal and immediately immersed in 2 % (w/v) ice-cold $OsO_4$ buffered to pH 7·4 with veronal acetate (Palade, 1952). They remained in the osmium for about 15 min. Afterwards the tissues were dehydrated by successive passages in alcohol (75–100 %). After dehydration the tissues were processed by Luft's method (1961): they were soaked in propylene oxide for 60 min and then passed into a mixture containing equal parts of epoxy resin (Epon 812, Shell) and propylene oxide. They remained in the mixture for 1–2 hr. Finally, they were embedded in the epoxy resin. The block obtained was trimmed and sectioned transversely with a Porter-Blum ultramicrotome. For observation and photography with the phase-contrast microscope (Carl Zeiss) the sections (1–2 $\mu$) were mounted on a glass plate, covered by a drop of Canada balsam and a microscope cover-slip. Observations with the electron microscope (Akashi-Bendix Tronscope) were performed on sections which had a silver or gold colour. Some preparations were stained with uranium acetate.

The myelinated nerve fibres were measured from enlarged phase-contrast photomicrographs (total magnification 1000–1500 times) and the non-myelinated fibres from enlarged electron micrographs (total magnification 4800–40,000 times). In both cases the photograph was laid flat (face upwards) on the stage of the Zeiss microscope. The eyepieces and objectives were removed. The microscope illuminator was turned on and the light spot was focused on the emulsion side of the picture. The diameter of the spot was adjusted by opening or closing the diaphragm of the illuminator. In all cases the spot diameter was first adjusted to cover the smallest fibres in the photograph (0·1 $\mu$ in the electron micrographs and 1·0 $\mu$ in the phase-contrast pictures) and the picture was scanned by manual displacement; whenever the area of a fibre clearly matched that of the light spot an electric counter was triggered and an ink mark made on the picture. Once all fibres of a given diameter were counted the procedure was repeated after increasing the diameter of the spot in steps of 0·1 $\mu$ for electron micrographs and of 1·0 $\mu$ for phase-contrast micrographs until all the fibres in a given photograph were measured. The diameter of fibres too large to be within the light-spot range was calculated by measuring two diameters at right angles and averaging the results obtained.

Physiological experiments were conducted *in vitro* in a manner similar to that already described (Eyzaguirre & Lewin, 1961*a*, *b*). In short, the carotid body and its own nerve in addition to the ganglio-glomerular connexions were excised from the animal and placed in a chamber through which flowed Locke's solution equilibrated with 100 % $O_2$ at pH 7·4. The saline was covered with mineral oil. The nerves or the whole preparation were lifted into oil for stimulation and recording.

## RESULTS

The carotid-body–sinus region, the carotid nerve and the ganglio-glomerular nerve were examined under a low-power stereo-microscope. The tissues under observation were cleaned from surrounding connective tissues and the course of the nerves followed.

The carotid nerve was followed to its termination on the carotid-body–sinus region and was found to divide into two branches shortly before reaching this region. One branch reached the carotid body, clearly going to innervate the glomerular tissues, while the other nerve branch reached the carotid sinus. This finding permits the possibility of selective severance of either nerve branch, although *in vivo* such a procedure is difficult owing to the dense vascularization of the region and because of the presence of abundant connective tissue around the bifurcation of the nerve.

Usually two or three nerves were found to link the superior cervical ganglion with the carotid-body–sinus area. One of them clearly went to the sinus while the others connected the ganglion with the glomus. A common arrangement was the presence of a relatively thick nerve which divided in two branches before reaching the glomus. One of the branches seemed to penetrate the glomerular capsule; the other coursed toward the glomus, bypassed the organ and joined the carotid nerve. Sometimes the nerve branch which joined the carotid nerve could be followed as a separate entity from the cervical sympathetic ganglion. Occasionally, still another nerve was found leaving the ganglion; it coursed toward the carotid body, which it bypassed, and then ran separate from the carotid nerve to innervate, apparently, other organs in the neck. At times this nerve could not be followed as a separate entity, since it originated as a branch of the ganglio-glomerular nerve.

## Physiological observations

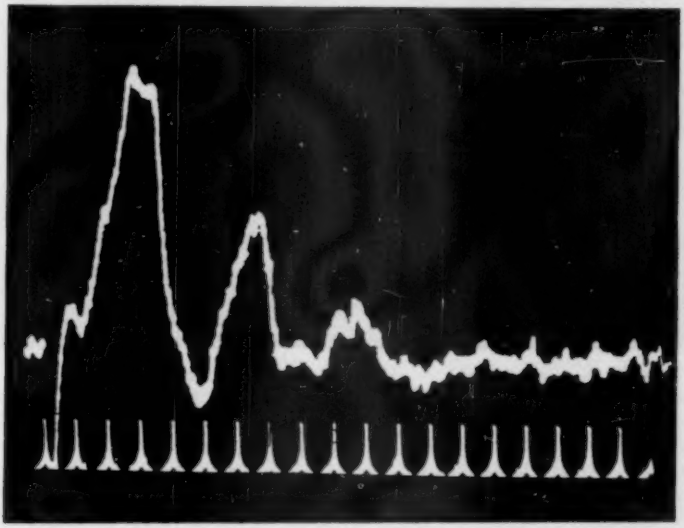
### *The non-myelinated fibres in the carotid nerve*

It is well known that the majority of myelinated fibres in the carotid nerve subserve either presso- or chemoreceptor functions. The presence of non-myelinated fibres in this nerve is less well established. The only available evidence is that of Douglas & Ritchie (1956), who found during stimulation and recording from the carotid nerve a potential conducted at C-fibre velocity. They estimated that the conduction velocity of the fibres responsible for the recorded potential was from 1·0 to 1·3 m/sec at 37° C.

The present series of experiments was designed to study further the C-fibre activity of the carotid nerve under conditions similar to those employed by Douglas & Ritchie. The glomus and the carotid nerve were lifted into oil. One stimulating electrode was placed on the glomus while the other was placed on the carotid nerve 1 mm from the first electrode; recording leads were placed at the other end of the nerve. Text-figure 1 illustrates such an experiment. In this particular instance the myelinated fibre potential was not recorded, presumably owing to injury. Maximal stimulating shocks elicited three distinct deflexions and the conduction

velocity was calculated as 1·75, 0·61 and 0·38 m/sec for the fibres responsible for the onset of the first, second and third C-fibre potential complexes.

The diameters of the fibres involved may be calculated by using Gasser's (1955) formula, i.e. dividing the conduction velocity by a factor of 1·7. With this method one may estimate that the first group of C fibres had a diameter of about 1·03 $\mu$, while the other two groups had diameters of 0·36 and 0·22 $\mu$ respectively. If the physiological results just described are

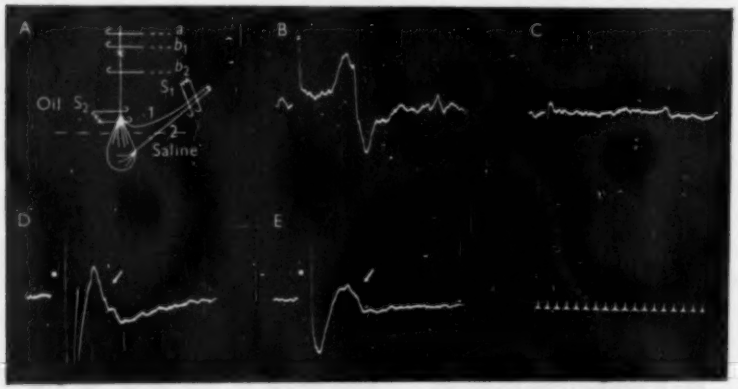


Text-fig. 1. Non-myelinated fibre potential complexes evoked in carotid nerve by stimulation of carotid nerve near glomus. Recording leads at other end of nerve. Distance between cathode and proximal recording electrode 3·5 mm. Temp. 36·8° C. Myelinated fibre activity was not recorded. Following artifact three non-myelinated fibre potentials may be seen. Time marker, 1 msec.

correlated with the diameter distribution curve of non-myelinated fibres in the carotid nerve (see Text-fig. 3A), it is clear that the first and larger potential complex was apparently elicited by fibres which are not abundant in the carotid nerve. The larger amplitude of this potential may have been due to several factors such as larger diameter of the fibres involved, less temporal dispersion and more pronounced summation of the potentials produced by individual fibres. The second and third potential complexes, conducted at 0·61 and 0·38 m/sec were, on the other hand, elicited by fibres (0·36 and 0·22 $\mu$) which are very numerous in the carotid nerve.

*Presence of non-sympathetic C fibres*

The results presented in the preceding section confirmed and extended Douglas & Ritchie's (1956) observations in the sense that C-fibre potentials may be detected in the carotid nerve upon stimulation. However, it remained uncertain whether or not C fibres other than those of sympathetic origin (Eyzaguirre & Lewin, 1961*b*) occurred in this nerve.




Text-fig. 2. Non-myelinated fibre potentials recorded from carotid nerve by stimulation of same nerve and of ganglio-glomerular connexions. A, anatomical arrangement and position of electrodes; glomus immersed in saline covered by paraffin oil, nerves lifted into oil for stimulation and recording. 1 and 2 represent two branches of ganglio-glomerular nerve; $S_1$, stimulating electrodes on ganglio-glomerular nerve; $S_2$, stimulating electrodes on carotid nerve; *a*, distal recording electrode on cut end of carotid nerve; $b_1$ and $b_2$, two different positions of proximal recording electrode. About 1·5 mm between *a* and $b_1$, and about 3·0 mm between *a* and $b_2$. In position $b_2$ recording electrode at about 1·5 mm from carotid body. B, carotid-nerve potential elicited by maximal stimulation of ganglio-glomerular nerve and recorded between *a* and $b_2$. C, disappearance of potential after moving proximal lead from $b_2$ to $b_1$. D, potentials evoked in carotid nerve by stimulation of same nerve by $S_2$; proximal recording lead at $b_2$. Myelinated fibre potential indicated by dot (most of it off screen); non-myelinated fibre potential marked by arrow. E, same as D, but this time proximal recording electrode at $b_1$ position. Time marker, 1 msec.

In this series the glomus and the carotid nerve in addition to the ganglio-glomerular connexions were set up for stimulation and recording. Care was taken to leave the carotid body in the saline equilibrated with 100 % $O_2$ in order to decrease the sensory discharges. Both the ganglio-glomerular and carotid nerves were lifted into oil for stimulation and recording. Such an experiment is shown in Text-fig. 2. A represents the anatomical situation and the position of the stimulating and recording leads. B shows the potential complex evoked in the carotid nerve by maximal shocks

applied through leads $S_1$ to the ganglio-glomerular nerve. The carotid-nerve potential was recorded by placing one recording electrode ($a$) at the cut end of the nerve while the second recording lead ($b_2$) was placed at 1·5 mm from the carotid body. The potential obtained was a C-fibre complex (cf. also Eyzaguirre & Lewin, 1961*b*). The proximal recording lead was then moved to $b_1$, and the previously obtained potentials disappeared as illustrated in C. Afterwards the proximal recording lead was moved back to position $b_2$, and the carotid nerve was stimulated by leads $S_2$. Results obtained are illustrated in D. Single nerve shocks elicited a large myelinated-fibre potential (most of it off the oscilloscope screen and marked by a dot) and a later deflexion (arrow) clearly produced by C-fibre activity. In E, the proximal recording electrode was moved back to position $b_1$, where again the large myelinated-fibre deflexion may be seen followed by a C-fibre complex, which this time was smaller than that obtained in D.

In the experiment just described it is not known why the C-fibre potential elicited by ganglio-glomerular stimulation was not recorded when the proximal recording electrode was moved from position $b_2$ to position $b_1$. A possible explanation is that a fortunate and selective injury of the carotid-nerve C fibres of sympathetic origin occurred at this point. In any case, the fact that a C-fibre potential was obtained while the carotid nerve was stimulated by $S_2$ leads and potentials recorded by leads at $a$ and $b_1$ positions clearly indicates that non-sympathetic C fibres occur in the carotid nerve.

At the end of the above experiment an effort was made to determine, in the same preparation, the pathway of sympathetic C fibres which are found in the carotid nerve. As is illustrated in Text-fig. 2A, the nerve linking the superior cervical ganglion with the carotid body divides into two branches, one going to the carotid body (branch 2) while the other bypasses the organ (branch 1) to join the carotid nerve at about 0·5 mm from the point where the carotid nerve penetrates into the glomus. The ganglio-glomerular nerves were stimulated and potentials recorded from the carotid nerve as illustrated in Text-fig. 2B. Immediately after obtaining the carotid-nerve potential of sympathetic origin, nerve branch 1 was severed and the potential previously obtained could no longer be recorded. This finding has been obtained also in other similar experiments. It may be concluded, therefore, that carotid-nerve sympathetic potentials are obtained from stimulation of sympathetic nerve fibres that join the carotid nerve near the carotid body and not from fibres that apparently penetrate into the glomus. The latter presumably correspond to those fibres described by de Castro (1926) which course in the interstitial spaces of the organ. These fibres may contribute to the vascular innervation of the organ and

be responsible for the small increase in chemoreceptor discharges that is obtained in the animal, apparently due to vasoconstriction (Eyzaguirre & Lewin, 1961*b*).

## Anatomical observations

A series of observations were made to study the fibre content of the carotid nerve, carotid body and ganglio-glomerular nerve by means of phase-contrast and electron microscopy. Results presented below show that the carotid nerve possesses numerous non-myelinated fibres besides the well known myelinated axons. They also show the distribution of both myelinated and non-myelinated axons within the carotid body and the diameter distribution of non-myelinated fibres in the ganglio-glomerular nerve.

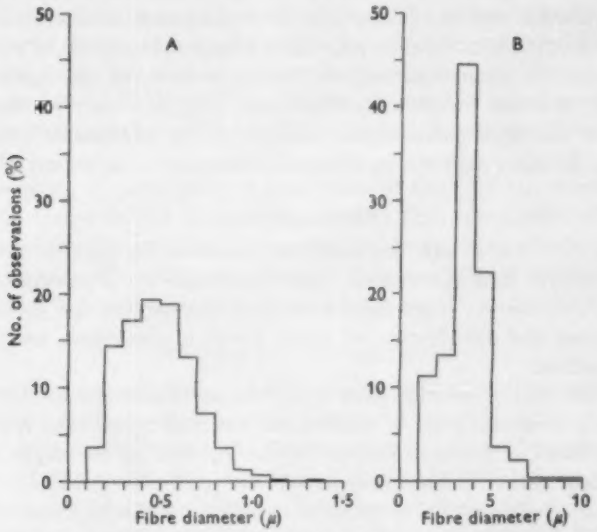
### *The carotid nerve*

Plate 1 A illustrates a cross-section of the carotid nerve near its central portion, as examined with the phase-contrast microscope; the abundance of myelinated fibres is evident. The spaces between the myelinated fibres are occupied by tissue bundles which are better seen in Pl. 1 B and C. These are phase-contrast micrographs obtained from carotid-body sections at the point of carotid-nerve penetration. The tissue bundles observed between the myelinated fibres correspond to groups of non-myelinated (C) fibres, as was evident on examining the specimens under the electron microscope. Plate 2 shows an electron micrograph obtained from a section of the carotid nerve near the glomus. It may be noticed that the myelinated fibres are outnumbered by the non-myelinated axons (arrows). The latter are grouped in bundles enveloped by Schwan-cell sheaths. In several instances the Schwan-cell nucleus (S) may be seen surrounded by groups of C fibres.

The diameter distribution of non-myelinated fibres was obtained from 30 sections derived from two different nerves. A total of 2037 non-myelinated fibres were measured and their diameter distribution curve is presented in Text-fig. 3 A. This unimodal curve shows that non-myelinated fibres in the carotid nerve have diameters ranging from 0·1 to 1·3 $\mu$, the mode of the frequency distribution being 0·3–0·5 $\mu$.

The myelinated fibres in the carotid nerve number from 600 to 700 fibres (de Castro, 1951). De Castro indicated that 17·5 % of these fibres had diameters of from 1·5 to 2·8 $\mu$; 79 % of the fibres from 3 to 5 $\mu$ and 3·5 % had diameters of 6–8 $\mu$. However, since no diameter distribution curve seemed to be available all the myelinated fibres (578) in one carotid nerve were measured. The diameters of these fibres ranged from 1·0 to 9·0 $\mu$ and their diameter distribution is presented in Text-fig. 3 B. From the illustration it may readily be seen that the values obtained from this nerve are similar to those presented by de Castro.

The proportion of non-myelinated fibres relative to myelinated ones was estimated indirectly, since the former could not be counted from the phase-microscope photographs and the electron micrographs did not include a sizeable portion of the nerve. Consequently, fifteen electron micrographs (total magnification 4800–8400 times) were used in the counting of




Text-fig. 3. Diameter distribution curves of fibres in the carotid nerve of the cat. A, measurements obtained from 2037 non-myelinated fibres analysed from 30 sections made in two nerves. B, measurements from all myelinated fibres (578) from one carotid nerve.

TABLE 1. Ratio of non-myelinated to myelinated fibres in the carotid nerve. Sections made at three different levels; at the origin of nerve in the glomus, at 1·0 mm from the origin and at the middle of nerve

| Section level | No. of non-myelinated fibres | No. of myelinated fibres | Ratio |
|---|---|---|---|
| Origin | 603 | 122 | 4·94 |
| 1 mm from origin | 725 | 160 | 4·53 |
| Middle | 404 | 191 | 2·11 |

myelinated and non-myelinated nerve fibres. The photographs were obtained from sections made at three different levels, namely, at the glomerular origin of the carotid nerve (same region as that illustrated in Pl. 1 B and C), at 1 mm from the glomerular origin and also at a point close to the middle of the carotid nerve. Table 1 shows the ratio of non-myelinated to myelinated fibres obtained at these three different levels.

From the Table it is clear that non-myelinated fibres are more numerous than myelinated ones. However, the ratio becomes smaller at the middle of the nerve than it is near the glomus. The number of fibres counted is small and, therefore, it is difficult to draw definite conclusions as to the reason for the reduction in the ratio of non-myelinated to myelinated fibres. A possible explanation is that near the middle of the carotid nerve the section includes both the glomerular and sinus branches of the nerve. It is possible that the sinus branch may have a larger proportion of myelinated axons than the glomerular branch. Serial sections of the region will be necessary in order to elucidate this point. The fact that in the carotid nerve the non-myelinated axons outnumber the myelinated ones is not unusual. In other nerves something similar occurs (Davenport & Ranson, 1931).

*The carotid body*

The glomus was serially sectioned starting from the point of entry of the carotid nerve, and specimens were examined by phase-contrast and electron microscopy. Particular attention was paid to the nerves in the organ, since the distribution of nerve fibres in the organ has not been clearly defined.

Near the pole of carotid-nerve entry the nerves are grouped in bundles containing large numbers of myelinated and non-myelinated nerve fibres (Pl. 1 B and C). Sections nearer to the equator of the organ revealed myelinated nerve fibres interspersed between the glomerular tissues. Sometimes it was possible to detect at the centre of the organ nerve bundles formed by myelinated and non-myelinated nerve fibres. Also, near the periphery of the equatorial region, well defined nerve bundles with both types of nerve fibres were clearly recognized. The centrally located nerve bundles became less conspicuous as sectioning proceeded toward the other pole of the organ, although the peripherally located bundles were still clearly seen.

Plate 3 A and B shows electron micrographs obtained from sections performed near the equatorial region of the carotid body. A shows a cross-section of a nerve bundle located at the periphery of such a region. A number of myelinated fibres (M) may be seen, some of which clearly show the Schwan-cell (S) nucleus. The Schwan-cell membrane of these fibres is distinctly seen in all instances. In addition, numerous non-myelinated fibres (arrows) may be seen, some of them around a Schwan-cell (S) nucleus; they clearly outnumber the myelinated axons. It is not known whether or not some (or all) of these fibres have a sympathetic origin. Plate 3 B shows a cross-section made at the same level as that shown in A, but this section covered the centre of the glomus. Two 'chief' cells (G) may be seen with their large nucleus (Gn); one of them is enveloped by a sustentacular

process (Sp) similar to those described by Ross (1959). The cell cytoplasm is abundantly supplied by mitochondria and by small osmiophilic granules, as described previously by other authors (Garner & Duncan, 1958; Lever, Lewis & Boyd, 1959). An interesting feature of this illustration is that myelinated (M) and non-myelinated (arrows) fibres are interspersed among the cells. The non-myelinated fibres may be either non-myelinated C fibres (either of medullary or sympathetic origin) or the non-myelinated endings of myelinated fibres. It is known that a number of myelinated fibres from the carotid nerve lose their myelin as they approach the glomerular cells (de Castro, 1951). If those fibres are responsible for the transmission of chemoreceptor impulses they would be similar to sensory fibres of other receptors.

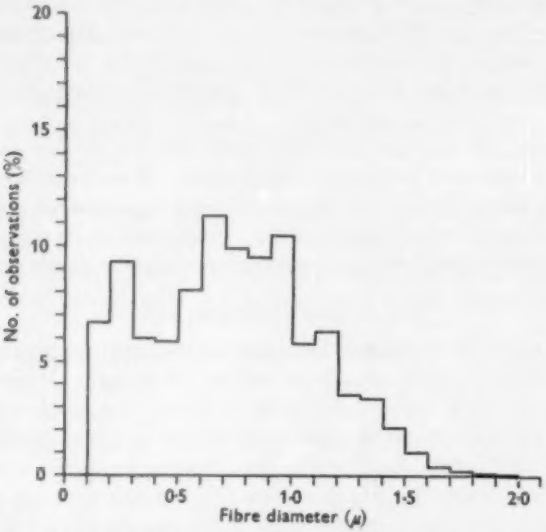
In summary, it may be said that cross-sections of the carotid body reveal the presence of numerous myelinated and non-myelinated fibres. These fibres tend to be rather dispersed and less numerous near the pole opposite to that of carotid-nerve entry. They tend to be more numerous and grouped in bundles as they approach the carotid-nerve pole.

### *The ganglio-glomerular nerve*

This nerve was also examined by phase-contrast and electron microscopy. The sections were made at a level where the ganglio-glomerular nerve appeared as a single bundle. At times, however, the section showed the presence of two distinct nerve bundles which corresponded to the two nerve branches of this nerve (see above). Both branches showed a similar type of fibre content, namely, very abundant C fibres and very few myelinated axons. Plate 4A shows a cross-section of the ganglio-glomerular nerve as examined with the light microscope. The nerve is well encapsulated and within the capsule numerous C fibres may be seen. Only very few myelinated fibres (marked by arrows) seem to be present in this nerve (about 30 in the photograph). A few blood vessels are also apparent in this picture. Plate 4B is an enlarged electron micrograph obtained from the same nerve for the purpose of showing the numerous non-myelinated fibres. These fibres are grouped in small bundles enveloped by the Schwan-cell membrane. A few Schwan-cell nuclei (S) may be seen surrounded by non-myelinated axons.

The non-myelinated fibres of the ganglio-glomerular nerve were measured from electron micrographs obtained from sections made at a point where the nerve had not yet divided into the two branches described before. A total of 2390 non-myelinated fibres were analysed from 26 sections obtained from two nerves. Their diameter distribution curve is presented in Text-fig. 4. The fibre diameters range from 0·1 to 2·0 $\mu$. The curve shows a possible tendency to bimodality with a peak at 0·2 $\mu$ and a second pro-

minence with a not-too-well-defined peak at 0·6 to 0·9 $\mu$. The section covered, in all likelihood, the nerve fibres going to the glomus in addition to those joining the carotid nerve without entering the carotid body (see above). It is possible, therefore, that a selective section of the sympathetic carotid-nerve branch may reveal a clear bimodal distribution, as one would expect from physiological recordings (see Physiological observations, p. 270; also Eyzaguirre & Lewin, 1961*b*).




Text-fig. 4. Diameter distribution of non-myelinated nerve fibres in ganglio-glomerular nerve. Measurements from 2390 fibres from 26 sections made in two nerves.

## DISCUSSION

De Castro (1951) has indicated, on the basis of histological evidence, that the larger carotid-nerve myelinated fibres probably convey pressoreceptor impulses, while the smaller ones are probably chemoreceptor fibres. This suggestion has been frequently accepted as valid, since carotid-nerve action potentials elicited by pressoreceptor fibres are usually larger than those evoked by chemoreceptor fibres. However, this is not always the case, since small action potentials may be elicited by pressoreceptor fibres (Landgren, 1952) and often it is possible to record action potentials from chemoreceptor fibres which may be larger than pressoreceptor ones (cf. Heymans & Neil, 1958). However, the available physiological evidence does not provide a definite answer to this question, since it is based on action-potential magnitude which is dependent on local recording condi-

tions. The anatomical evidence presented in this study does not by itself solve this problem even if large myelinated fibres may be found in the carotid body (see Pl. 1B and C); de Castro (1926, 1951) has shown that myelinated fibres penetrate the glomerular capsule to innervate the arteries and arterioles of the glomus. Conduction-velocity studies are necessary in order to determine the relative diameters of presso- and chemoreceptor fibres present in this nerve. In this connexion it is worth noting that Paintal (1953) has shown that aortic chemoreceptor fibres conduct at 7–12 m/sec while pressoreceptor fibres conduct at 12–53 m/sec, the assumption being that the former are smaller than the latter.

The above picture is complicated by the finding of numerous non-myelinated fibres in the carotid nerve. Some of these fibres originate from the superior cervical ganglion, but a number of them have, presumably, an intracranial origin. The functional role of either type of C fibre is unknown. It is possible, however, that some C fibres of cranial origin may subserve either sensory or efferent functions and participate in reflex responses originating either in the carotid body or sinus. Any discussion concerning the possible role of the carotid-nerve sympathetic C fibres will have to wait until the central connexions of this pathway are determined.

The finding of numerous C fibres in the carotid nerve of the cat brings into focus the observation of Douglas & Ritchie (1956), who found that cardiovascular reflexes may be elicited in the rabbit upon stimulation of carotid-nerve C fibres. But it is not known whether the carotid nerve of the rabbit has more than one type of non-myelinated fibre as is the case in the cat. Consequently, until this point is established it will not be clear whether they stimulated sympathetic C fibres or other non-myelinated axons.

## SUMMARY

1. The innervation of the carotid body and the fibre content of nerves reaching this organ was studied by examining fresh specimens under a dissecting microscope and polarized light, by stimulation and recording from the nerves and by studying fixed specimens under phase-contrast and electron microscopes.

2. Examination of fresh tissues showed that the carotid nerve divides near the glomus into two branches: one goes to the sinus while the other innervates the glomus. The glomerular branch undergoes further subdivisions in order to innervate different glomerular lobules. Usually two or three nerves link the superior cervical ganglion with the carotid-body-sinus area. One nerve goes to the sinus while one or two reach the carotid body. From the latter a nerve branch leaves the nerve close to the glomus to join the carotid nerve, bypassing the carotid body.

3. Physiological experiments have shown the presence of two types of non-myelinated fibres in the carotid nerve; the fibres of one type come from the superior cervical ganglion and travel in the carotid nerve via the branch that bypasses the glomus. Other non-myelinated fibres have, presumably, an intracranial origin.

4. The fixed specimens showed that the carotid nerve possesses abundant myelinated fibres and very numerous non-myelinated axons which outnumber the myelinated fibres. The non-myelinated axons have diameters ranging from 0·1 to 0·3 $\mu$ and the myelinated ones of from 1·0 to 9·0 $\mu$. Serial sections of the carotid body indicate the presence of numerous myelinated and non-myelinated fibres within the organ capsule. Some of these fibres are interspersed among the glomerular cells while others are grouped in bundles near the periphery of the organ. Fibre grouping tends to become more conspicuous near the pole of carotid-nerve penetration. The ganglio-glomerular nerve(s) consists mainly of non-myelinated fibres with diameters ranging from 0·1 to 2·0 $\mu$; myelinated fibres are few.

5. The possible role of different fibres in the carotid nerve is discussed.

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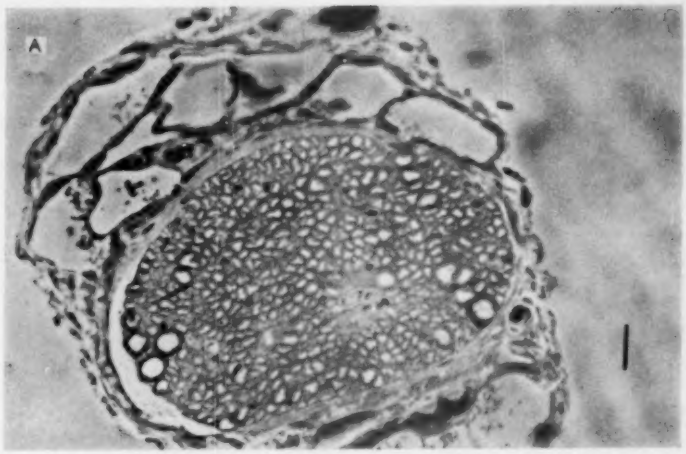

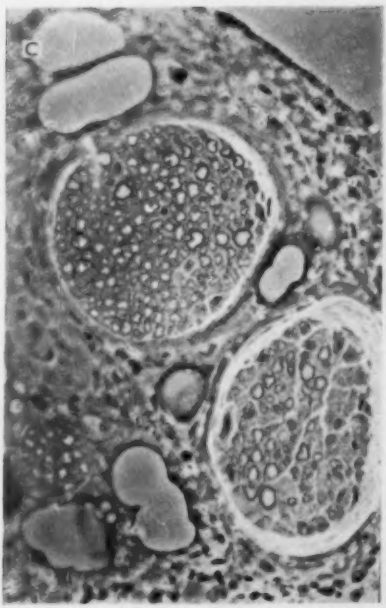


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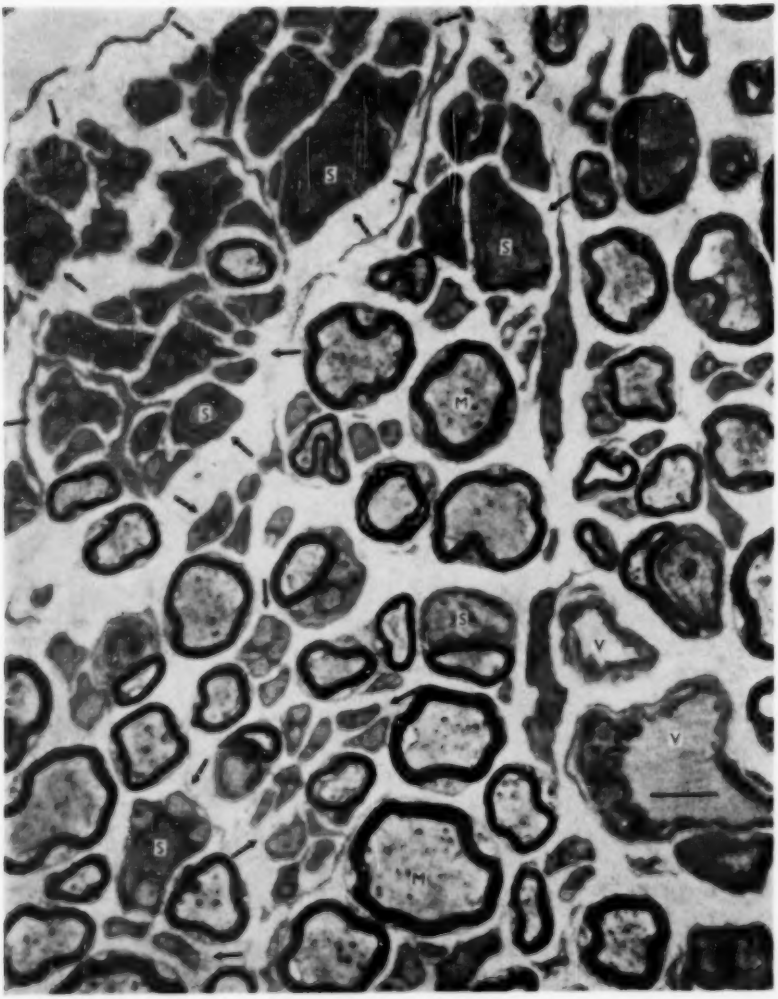


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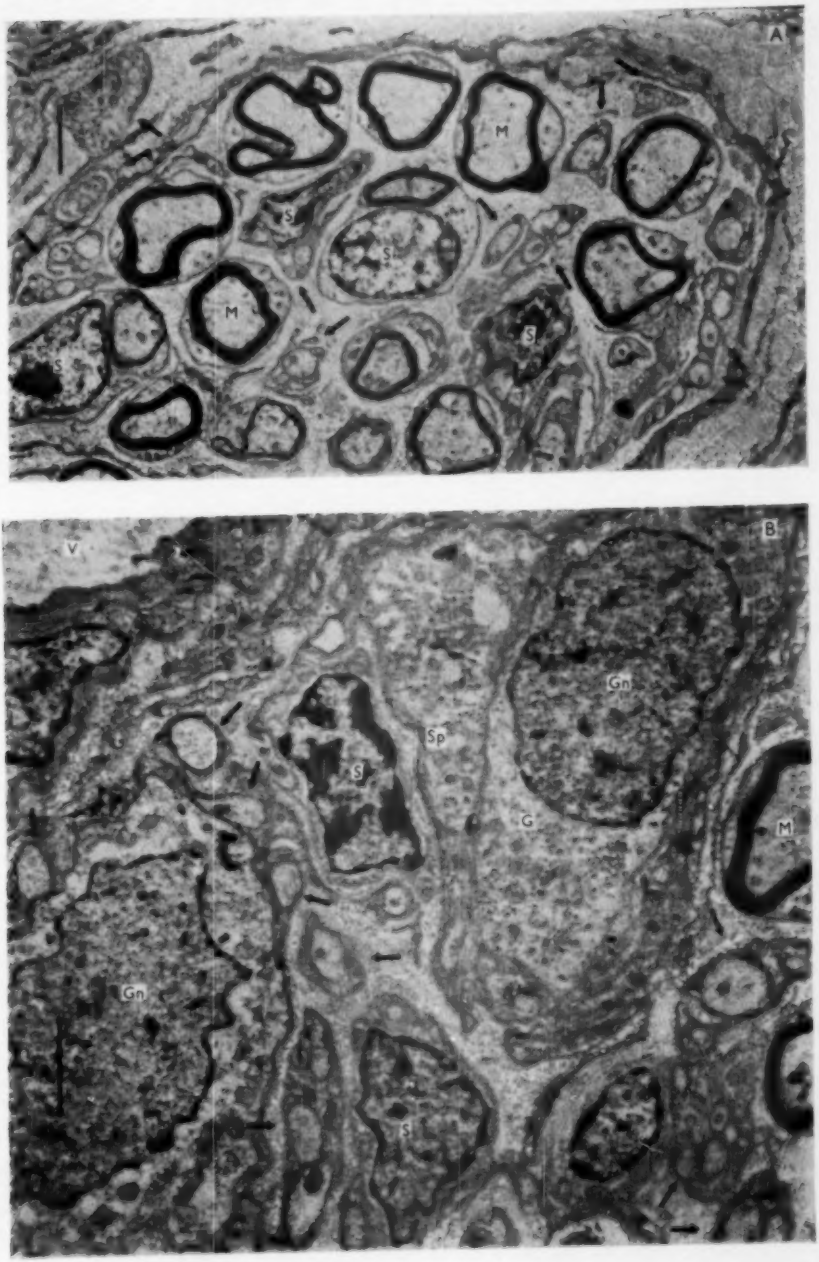


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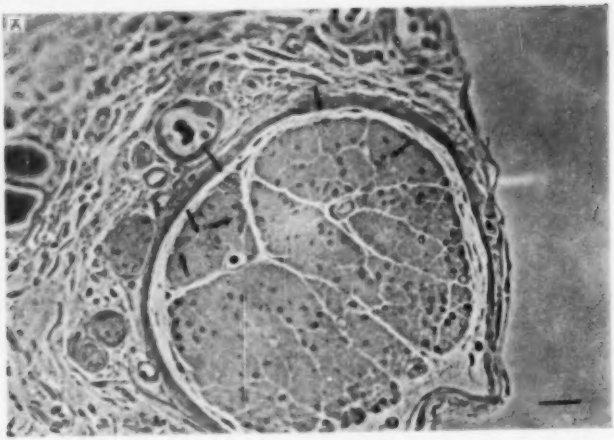

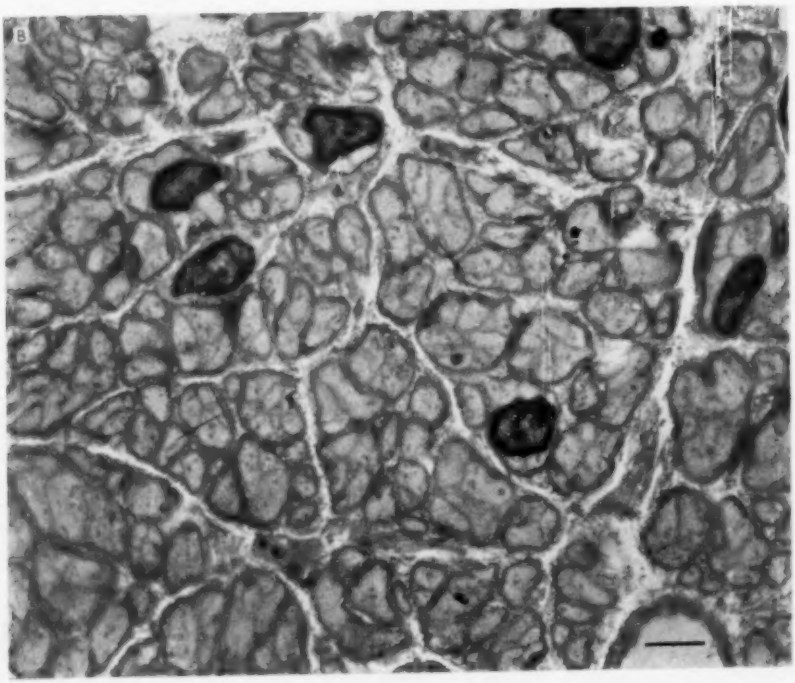

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## EXPLANATION OF PLATES

### PLATE 1

Phase-contrast micrographs of carotid nerve in the cat. A, cross-section near middle of nerve. B and C, cross-sections of glomus at point of carotid nerve penetration. Note the different nerve bundles. Vertical bar in A = 14·1 $\mu$.

### PLATE 2

Electron micrograph obtained from carotid-nerve section (unstained) near its point of origin. Note myelinated fibres (M) and numerous C-fibre groups indicated by arrows. Sometimes clusters of C fibres are surrounding a Schwan-cell nucleus (S). At the lower right-hand corner two capillary vessels may be seen (V). Horizontal bar = 2 $\mu$.

### PLATE 3

Electron micrograph obtained from cross-section at the middle of the carotid body; specimen stained with uranium acetate. A, section of nerve bundle near glomerular capsule. Several myelinated fibres (M) outnumbered by non-myelinated axons (arrows). Some Schwan cells (S) envelop either myelinated or non-myelinated axons. B, cross-section of glomus at centre of organ. Two myelinated fibres (M) may be seen at right-hand edge of picture. Two 'chief' cells (G) are present, showing their large nuclei (Gn). The upper 'chief' cell is enveloped by a sustentacular process (Sp). Numerous non-myelinated fibres (arrows) may be seen interspersed among the cells. Three Schwan cells (S) are clearly seen. At the upper left corner a blood vessel (V) is seen. Vertical bars = 2 $\mu$.

### PLATE 4

Cross-sections of the ganglio-glomerular nerve. A, phase-contrast micrograph showing most of the nerve which is formed mainly by non-myelinated fibres. Arrows indicate small clusters of myelinated fibres. Horizontal bar = 15 $\mu$. B, electron micrograph from section of the same nerve, stained with uranium acetate. Note clusters of C fibres. The dark bodies represent Schwan-cell nuclei. Horizontal bar = 2 $\mu$.

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# THE DISCHARGE PATTERN OF MUSCLE SPINDLES OF THE RABBIT ON ACTIVATION OF INTRAFUSAL MUSCLE FIBRES

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Histological studies show two types of receptor ending in the muscle spindle. The one has been called annulospiral, nuclear-bag, A 2 or primary; the other has been called flower-spray, myotube, A 1 or secondary. It is generally agreed that the primary endings have larger nerve fibres than the secondary endings, and Hunt (1954) showed that the receptor fibres of the muscle spindle were bimodally distributed, one peak lying in Group I and the other in Group II. There has since been a tendency to classify all spindle receptor fibres conducting at Group I velocity (72–120 m/sec) as coming from primary receptors, and those conducting at Group II velocity (24–72 m/sec) as coming from secondary receptors.

When the intrafusal muscle fibres alone contract the frequency of discharge of the spindle receptors is increased. Moreover, Hunt (1954) showed that there was no qualitative difference in behaviour between the two types of spindle receptor when the intrafusal muscles were made to contract by repetitive stimulation of the small motor fibres in the ventral roots. When the extrafusal muscle fibres alone contract, the strain is removed from the muscle spindles by virtue of their arrangement in parallel with the extrafusal fibres (Fulton & Pi-Suñer, 1928). The spindle receptors therefore stop discharging during the rising phase of the muscle tension record (Matthews, 1933). When both the intrafusal and extrafusal muscle fibres contract, as happens when strong stimuli are applied to the muscle nerve, the resultant of their contractions is such that some endings do not stop discharging during the rising phase of the muscle tension record (Matthews, 1933; Hunt & Kuffler, 1951). These endings are supplied by fibres of fast conduction and hence it is likely that they are primary endings. The response of the secondary endings during supramaximal stimulation of the muscle nerve has, however, remained uncertain. Matthews (1933) showed that certain muscle spindle endings, the A 1 endings which were believed to be identical with secondary endings, could not be activated during contraction of the extrafusal muscle (see also Cooper

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1959). Hunt (1954), however, showed that some of the endings with fibres of Group II conduction velocity could be activated by muscle nerve stimulation when the muscle contracted isometrically. In view of these divergent results, it was considered necessary to re-investigate the effect of extrafusal and intrafusal activity on the response of secondary muscle spindle endings.

In this paper, an attempt has been made to provide an explanation of differences in behaviour of muscle spindle receptors, and to discover the conditions in which both types of receptor ending may be activated. It has been found that most of the lower velocity spindle receptor fibres are activated by stimulation of the muscle nerve only if the contraction of the extrafusal muscle fibres is removed or reduced, and when the stimulation is tetanic. If, as seems probable, these fibres are from secondary endings, then the work of Boyd (1959) leads one to suggest that the secondary endings of the muscle spindle are influenced by slow muscle capable of graded contraction, whereas the primary endings lie mainly on muscle capable of all-or-nothing twitches.

A preliminary account of this work has already appeared (Diete-Spiff, 1960).

## METHODS

Rabbits were anaesthetized either with a 5% solution of sodium hexobarbitone or with urethane (25g/100 ml.) in 0·9% NaCl solution. When urethane was used, the full dose (1·6–1·8 g/kg body weight) was given into a marginal ear vein. Further doses were administered intraperitoneally as required. When sodium hexobarbitone was used, sufficient was administered initially into a marginal ear vein to induce surgical anaesthesia, and thereafter surgical anaesthesia was maintained by small doses administered through a polythene cannula in the right femoral vein. After the trachea had been cannulated, the left leg was partially denervated by cutting the femoral and obturator nerves, and 5–10 mg of hexamethonium bromide (in 0·9% NaCl) given intravenously to lower the blood pressure. This enabled a relatively bloodless laminectomy to be performed. The blood pressure returned to normal inside 30 min, well before the start of the experiment proper. The cord was usually exposed from the level of L 5 to S 2 spinous processes. The animal was then fixed rigidly by clamps at the upper ends of both femoral bones, a clamp at the lower end of the left tibia, and a pin through the muscles and spinous processes of the 4th lumbar vertebra. An incision was made in the left popliteal fossa extending inferiorly to the lower limits of this fossa and superiorly to the level of the first sacral spinous process. All the branches of the sciatic and popliteal nerves were then cut, except the nerve to the medial head of the gastrocnemius muscle and the nerve to the plantaris muscle of the left leg. Both the triceps surae tendon and that of the plantaris muscle were prepared for recording tension. After isolating a receptor fibre the muscle containing its ending was identified by stretching it; the nerve to the other muscle was then cut. The tendon of the semi-membranosus muscle was cut through the same incision.

In order to limit the field of action of drugs which were to be injected intra-arterially later in the experiment, the side branches of the femoral and popliteal arteries were tied off as they were revealed during various stages of the dissection. After the tendons had been prepared, the posterior tibial artery was cannulated with a fine heparinized cannula, and the anterior tibial artery tied off at the level of the ankle.

The dura mater was opened under warm paraffin oil (B.P.), equilibrated with water, at 37–38° C. The ventral and dorsal roots from the L 6 to S 1 (at times also L 5) were cut on the left side, after which L 7 or S 1 dorsal root was mounted on a single platinum electrode. The spindle receptor discharges were sampled in split filaments of one of these two dorsal roots, almost always from S 1. The muscle was made to contract by stimulating its nerve. The stimuli used in all instances were of 120 $\mu$sec duration, and for tetanic stimulation the frequency was 150–200/sec. The action potentials as well as the tension changes were amplified and recorded by means of a conventional amplifying system. The conduction velocity was calculated from a measurement of conduction time from the stimulus artifact to the start of the evoked spike and the conduction distance, which was measured after killing the animal, without removing the nerve from the body. No correction was made for setting-up time at the stimulating electrodes. The action potentials set up by electrical stimulation were compared in amplitude and duration with those obtained by stretching the muscle (cf. criteria of Paintal, 1953). The experiments were performed at the initial tension which permitted the development of maximum tension. While stimulating the muscle nerve supramaximally with single nerve shocks, the initial length of the muscle was adjusted until the deflexion on the screen of the oscilloscope which signified tension appeared maximal; the experiment was then continued at that initial tension. The muscle and nerve temperatures were kept at 34–36° C, the rectal temperature at 37–39° C.

When succinylcholine chloride (Scoline; Allen and Hanbury) was used, it was given in Locke's solution by retrograde injection into the posterior tibial artery without occluding the circulation. The success of the method depends, among other things, on a relatively slow rate of injection together with a good blood pressure. Up to six doses of succinylcholine were injected into any one muscle, but the usual number of injections was from three to four. Thirty minutes at least was allowed to elapse between injections. The site of action of the drug is almost completely localized to the gastrocnemius, soleus, and plantaris muscles, as revealed by intra-arterial injection of Indian ink through the same artery before the animal was killed.

Some experiments were performed on rabbits spinalized at the level of the fifth lumbar spinous process.

*Nomenclature.* 'Spindle receptor fibre' is used in the text to include any nerve fibre from the muscle spindle which conducts impulses towards the spinal cord. It is synonymous with spindle afferent or sensory fibre of other writers. 'Slack muscle' means a muscle which has been freed from its insertion and is not attached to the myograph.

## RESULTS

The analysis is based on the discharges in twenty-eight spindle receptor fibres belonging to the gastrocnemius medialis or plantaris muscles. Any fibre whose frequency of discharge slowed or stopped during the rising phase of a just-maximal twitch of the muscle containing its ending was classified as a spindle receptor fibre; fibres whose frequency of discharge increased in similar circumstances were taken to be tendon organ receptor fibres (cf. Matthews, 1933).

It soon became clear that many muscle spindle receptors could not be induced to discharge during the rising phase of the muscle tension record in a maximal twitch. An attempt was therefore made to activate the muscle spindle receptors while stimulating the mixed nerve, but without the complication of extrafusal contraction. This was achieved by reducing or abolishing extrafusal muscle contraction with the drug succinylcholine

chloride, injected intra-arterially into the gastrocnemius and plantaris vessels. Since the administration of succinylcholine chloride forms an essential part of the experiments, its action on extrafusal muscle fibres and on muscle spindles will first be described.

### *The effect of succinylcholine on extrafusal contraction and on the discharge of spindle receptors*

Within a minute of intra-arterial injection of 25–500 $\mu$g of succinylcholine chloride, paralysis of the extrafusal muscle fibres ensued, the completeness of the paralysis depending on the dose. The degree of reduction in extrafusal tension was observed by stimulating the muscle nerve once every 4–5 sec at the stimulus strength which had previously caused maximal contraction of the extrafusal muscle fibres: recovery from paralysis was studied in a similar fashion. When an adequate stimulus was applied to the muscle nerve during the period of reduced or absent extrafusal contraction, spindle receptor discharges which were previously absent during the rising phase of tension occurred (Fig. 1). Thus the end-plates on the intrafusal muscle fibres are more resistant to succinylcholine than

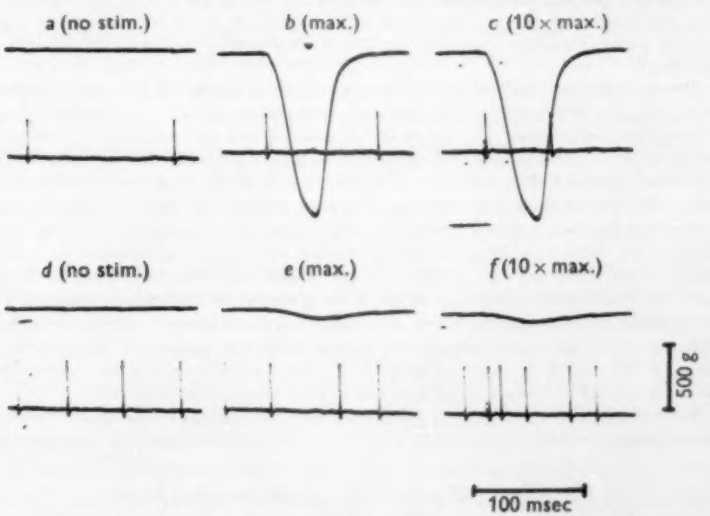


Fig. 1. Selective activation of a spindle receptor (3) (fibre conduction velocity of fibre = 95 m/sec) by single muscle nerve shock after paralysis of the extrafusal muscle fibres. Upper traces are tension records (increase in tension signalled downwards); lower traces are spindle receptor discharges. Plantaris receptor; urethane anaesthesia. *a–c*, before succinylcholine; *d–f*, after 100 $\mu$g succinylcholine. Note acceleration of the receptor discharge in *d* and discharges during contraction in *f*.

are those on the extrafusal muscle fibres. The dose of succinylcholine which was needed to produce extrafusal paralysis varied from 25 to 500 $\mu$g. This variation might have been caused by several factors, but was presumably due in part to the difference in the degree of localization of the drug: in most experiments 50–100 $\mu$g of succinylcholine was sufficient. By using a larger dose of succinylcholine than was necessary to block extrafusal contraction the intrafusal contraction could also be blocked. However, it was always possible to find a dose of succinylcholine which would block the extrafusal muscles selectively, thereby enabling the intrafusal muscle fibres to be activated on their own.

It was shown by Granit, Skoglund & Thesleff (1953) that succinylcholine excited muscle spindle receptors of the cat but had no effect on tendon organs of the same animal. The action of succinylcholine on similar receptors of the rabbit investigated in this work is in agreement with their findings. Two stages in the action of the drug, an initial and a later phase, may however, be differentiated.

In the initial phase the muscle showed irregular contractions ('fasciculation') which coincided with the time of arrival of the drug. The irregular contractions were clearly visible and tension changes of 100 g or more were recorded, especially in decerebrate animals. During this phase previously tonically discharging muscle spindle receptors stopped firing. In contrast, previously silent tendon organs started to discharge. The effect was presumably due to the fact that the drug caused the extrafusal muscle fibres to contract, thereby unloading the muscle spindle but producing sufficient tension to excite the tendon organs. The initial phase of succinylcholine action lasted a few seconds and led abruptly into the second stage.

In the second phase, that of spindle excitation, the discharge of the spindle receptors built up to a peak within 30 sec and sometimes persisted for 2 min or longer, depending on the dose of succinylcholine. Excitation which lasted over 5 min was seen. The effect was observed usually with 25 $\mu$g and invariably with 50 $\mu$g of succinylcholine. Spindle receptors whose fibres conducted at Group I as well as those whose fibres conducted at Group II conduction velocity were excited by the drug (Fujimori, Tokizane & Eldred, 1959). The receptors were still sensitive to stretch during and after the stage of excitation, but in some cases, especially with doses over 50 $\mu$g of succinylcholine, excitation was followed by complete silencing of the receptor. In a spindle receptor whose fibre was conducting at 36 m/sec 100 $\mu$g of succinylcholine caused excitation of the receptor, but this stopped abruptly 3 min after injection of the drug. At this time the receptor was insensitive to stretch, although stretch sensitivity soon returned and the normal behaviour pattern of the receptor was established. The blocking of the receptor by succinylcholine supports the suggestion by Granit *et al.* (1953) that the drug acts on the sensory terminals themselves.

In none of five tendon organs studied was excitation observed in the second phase, the phase of spindle receptor excitation.

### *The discharge pattern of spindle receptor fibres on stimulation of the muscle nerve*

In the later experiments the procedure was standardized as follows: After separation of a spindle receptor fibre, the conduction time from the stimulating electrode on the muscle nerve to the recording electrode on the dorsal root filament was recorded. The muscle nerve was then stimulated with single shocks of gradually increasing intensity until the twitch tension

reached a maximum. The intensity of the stimulus necessary to cause a maximal twitch will be referred to as the alpha-maximum voltage throughout the remainder of this paper. The responses of the spindle receptor fibre were observed whilst the alpha-maximum voltage was being determined. In no instances were there action potentials during the rising phase of the muscle twitch when the stimulus to the muscle nerve was below the alpha-maximum voltage. The stimulus intensity was then raised progressively up to ten times the alpha-maximum voltage. If discharges occurred during the rising phase of tension, the threshold voltage at which this took place was determined and expressed as a multiple of the alpha-maximum voltage. The stimulus strength was then set at the alpha-maximum voltage and the muscle nerve stimulated at a frequency of 150–200/sec. Again the stimulus intensity was raised progressively to ten times the alpha-maximum voltage; the threshold voltage at which spindle receptor discharges occurred during the rising phase of tension was determined. Succinylcholine was then injected; as stated previously this excited the receptors in addition to paralysing the extrafusal contraction. When this excitation was seen to be declining, i.e. in about 2 min from the time of injection of the drug, the muscle nerve was stimulated, with single shocks at intensities ranging from the alpha-maximum to ten times this voltage. If spindle receptor discharges occurred during the time, which in the absence of extrafusal paralysis would have been the rising phase of muscle tension, the threshold intensity of muscle nerve stimulation necessary to produce these discharges was determined. The procedure was then repeated, using tetanic stimulation, either immediately after the single shock testing or after a succeeding dose of succinylcholine. At the start of the period of testing the responses of the muscle and its receptor the latter was still obviously excited by the succinylcholine; later in the testing period the excitation was no longer apparent, i.e. the frequency of discharge of the receptor was now at its pre-injection level. The receptor may, however, have been still under the influence of the drug at this stage. In two experiments, therefore, gallamine triethiodide (Flaxedil; May and Baker) was used instead of succinylcholine. Gallamine paralyses the extrafusal muscle with no concomitant excitation of the spindle receptors. This is referred to again in the Discussion. The procedure detailed above was not followed in all experiments owing to the relatively short life-time of a receptor fibre after isolation. In the later experiments, therefore, if spindle discharges occurred during the rising phase of the muscle tension record to single shocks to the muscle nerve, no time was spent in stimulating tetanically. Instead, the threshold of a spindle receptor activation was determined repeatedly.

The findings are summarized in Table 1. The receptors fell into two

TABLE 1. Gastrocnemius medialis and plantaris spindle receptor fibres

| No. | Conduction velocity (m/sec) | Activated by single stimuli to muscle nerve | | Activated by tetanic stimulation of muscle nerve | | Threshold of activation (V) | Early discharge | Spontaneous discharge | Anaesthetic |
|---|---|---|---|---|---|---|---|---|---|
| | | Before *SCh* | After *SCh* | Before *SCh* | After *SCh* | alpha-maximum (V) | | | |
| 1 | 112 | + | + | *n* | + | 1·1 | + | − | *U* |
| 2 | 106 | + | *n* | + | *n* | < 10 | + | + | *SH* |
| 3 | 95 | − | + | *n* | *n* | < 10 | − | − | *U* |
| 4 | 91 | + | + | *n* | + | 3 | − | − | *U* |
| 5 | 88 | + | + | *n* | *n* | 2·2 | − | + | *U* |
| 6 | 87 | + | *n* | + | *n* | 1 | − | − | *U* |
| 7 | 85 | + | *n* | *n* | *n* | 1·1 | − | − | *U* |
| 8 | 80 | − | + | *n* | *n* | 2 | − | − | *SH* |
| 9 | 75 | + | *n* | *n* | *n* | 3·5 | − | 0 | *U* |
| 10*X* | 75 | − | + | *n* | − | < 10 | − | − | *U* |
| 11 | 74 | − | *n* | − | *n* | − | + | − | *U* |
| 12 | 74 | − | *n* | − | *n* | − | + | − | *U* |
| 13 | 70 | − | *n* | + | *n* | 3 | + | + | *SH* |
| 14 | 58 | − | − | − | + | 10 | − | + | *U* |
| 15 | 57 | − | − | − | + | 2·6 | − | + | *U* |
| 16 | 56 | − | − | − | + | 0·42 | − | + | *U* |
| 17 | 52 | + | + | − | *n* | 1·3 | − | + | *U* |
| 18*X* | 50 | − | + | − | + | 2·6 | − | + | *U* |
| 19 | 48 | − | + | − | + | 2·5 | − | + | *U* |
| 20 | 48 | − | *n* | + | *n* | 1 | − | + | *U* |
| 21 | 48 | − | *n* | − | *n* | − | − | − | *U* |
| 22 | 48 | − | *n* | − | *n* | − | − | − | *U* |
| 23 | 42 | − | − | + | *n* | 1 | − | + | *SH* |
| 24 | 41 | − | *n* | − | *n* | − | − | + | *U* |
| 25 | 40 | − | *n* | − | *n* | − | − | + | *U* |
| 26 | 36 | − | − | − | + | 0·13 | − | + | *U* |
| 27 | 36 | − | − | − | + | 8·5 | − | + | *U* |
| 28 | 33 | − | *n* | − | *n* | − | − | + | *U* |

*X*, Gallamine, not succinylcholine; *n*, not done; 0, not known; *SCh*, succinylcholine; *U*, Urethane; *SH*, Sodium hexobarbitone.

groups, namely, 13 which were activated by single shocks to the muscle nerve and 8 which were only activated when the muscle nerve was stimulated tetanically.

*Spindle receptors activated by single shocks to the muscle nerve.* Eight receptors responded to single nerve shocks by giving discharges during the rising phase of the twitch (Fig. 2*d* and *f* large spike). Five more fibres responded to single muscle nerve shocks when the extrafusal contraction was reduced or abolished with succinylcholine or gallamine. Thus a total of thirteen spindle receptor fibres could be activated by single shocks to the muscle nerve.

Eight out of these thirteen were also tested with tetanic stimulation. Two of these (18 and 19) had responded to single shocks only after treatment with succinylcholine, and these likewise responded to tetanic stimulation only after the drug had been given. Four others (1, 2, 4 and 6) also responded to tetanic stimulation (for details see Table 1) but the other two (10 and 17) showed a phenomenon which was unexpected. They were activated after succinylcholine by single but not by tetanic stimulation of the muscle nerve. No experiments were undertaken to discover the cause of this apparently anomalous finding. One possible cause, however, might be the type of depression described by Paintal (1959) and thought by him to be due to stimulation of spindle receptor fibres.

Figure 3*a* is a histogram showing the number of spindle receptor fibres plotted against conduction velocity. Those which could be activated by single shocks to the muscle nerve are shown cross-hatched. It is clear from this figure that almost all the fibres of fast conduction velocity (more than 70 m/sec) and a minority of those of slow conduction could be driven by single stimuli to the muscle nerve.

*Spindle receptors activated only by repetitive stimuli to the muscle nerve.* Some fibres which were not activated by single shocks to the muscle nerve were activated by tetanic stimulation of the nerve. All the fifteen fibres which were not activated by single supramaximal shocks to the muscle nerves were subjected to repetitive stimulation of the muscle nerve. In three of these fibres (13, 20 and 23), it was possible to set up spindle receptor discharges during contraction with stimuli to the muscle nerve from just maximal to three times the alpha-maximum voltage. In five more fibres activation by this means only occurred when the extrafusal contraction was diminished or abolished in the manner described previously. As extrafusal recovery advanced it was at times necessary to increase the number of stimuli necessary to activate the receptor (Fig. 4).

The fibres activated only by tetanic stimulation of the muscle nerve are shown in Fig. 3*b*. This group of fibres all had conduction velocities in the range 30–70 m/sec.

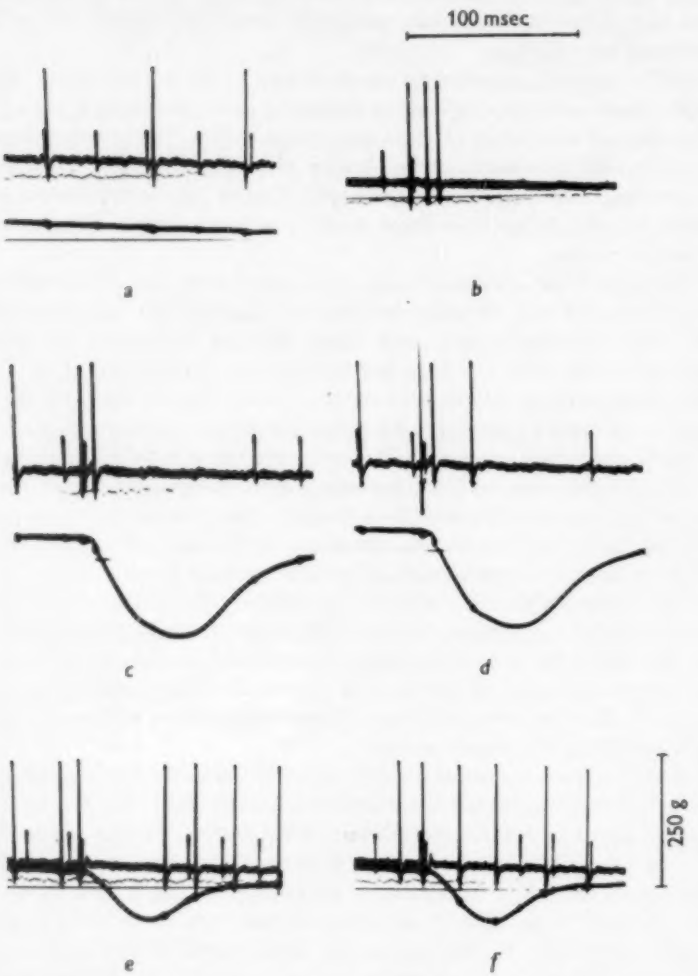


Fig. 2. Spindle receptor discharges during the rising phase of the twitch. Plantaris receptor; spinal rabbit under urethane anaesthesia. The large spike (1) was recorded from a fibre conducting at 112 m/sec, the small spike (19) from a fibre conducting at 48 m/sec. *a*, control, no stimulus; *b*, stimulus strength = alpha-maximum voltage; the muscle was slack; note 'early discharge'; *c*, stimulus strength = alpha-maximum; *d*, stimulus strength = 10 × alpha-maximum; *e*, alpha-maximum stimulus after 25 $\mu$g succinylcholine; *f*, 1·1 alpha-maximum stimulus after 25 $\mu$g succinylcholine. The slowly conducting receptor fibre was activated at 2·5 times the alpha-maximum voltage. Lower traces are tension records (increase signalled downwards); upper traces are receptor discharges.

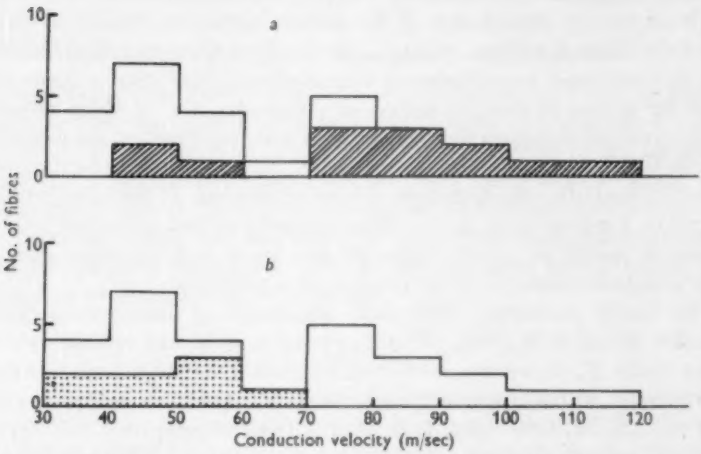


**Fig. 3.** Spindle receptor fibres activated by single shocks or by tetanic stimulation. ☐ All fibres. ▨ Fibres activated by single shock to muscle nerve with or without succinylcholine. ▤ Fibres activated only by repetitive stimulation with or without succinylcholine.

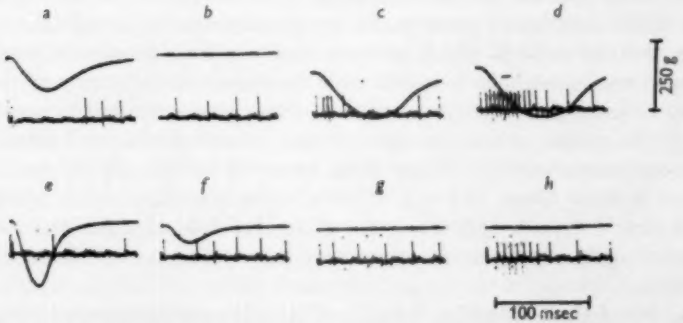


**Fig. 4.** Activation of a muscle spindle receptor (16) (fibre conduction velocity = 56 m/sec) by tetanic stimulation of the muscle nerve. Plantaris receptor; spinal rabbit under urethane anaesthesia. Records *a–d* form part of records taken at 5 sec intervals 7·5 min after 500 $\mu$g succinylcholine stimulating at 5·8 times alpha-maximum voltage: *a*, single stimulus; *b*, no stimulus; *c*, 4 stimuli, one response; *d*, 10 stimuli, three responses. Note the effect of increasing the number of stimuli. *e–h* form part of records taken at 5 sec intervals about 22 min after *a*, stimulating at 8·3 times alpha-maximum voltage synchronous with the injection of 300 $\mu$g succinylcholine: *e–g*, single shocks, *h*, tetanic stimulation.

*Fibres which were not activated.* Seven receptors were not activated by single or tetanic stimulation of the muscle nerve by stimuli up to ten times the alpha-maximum voltage. In all seven fibres extrafusal contraction was maximal; in no instances were the extra fusal muscle fibres paralysed by means of succinylcholine or gallamine. Six of these receptors were investigated before the technique of selective block of the extrafusal muscle fibres was fully developed (11, 21, 22, 24, 25, 28); the remaining fibre (12) died after the first dose of succinylcholine. If the selective block technique has been used, the receptors might have been activated by single shocks or repetitive stimuli to the muscle nerve, but this suggestion can only remain conjectural in the present circumstances.

*The 'early discharge'.* The early discharge of mammalian muscle spindles (Hunt & Kuffler, 1951) was seen in only five spindle receptor fibres (Table 1). It was easiest to demonstrate when the muscle was slack. The receptor fibres in which it occurred were all of high conduction velocity (70, 74, 74, 106, 112 m/sec). This is in agreement with the observations of Granit, Pompeiano & Waltman (1959), who found the early discharge almost exclusively in spindle receptor fibres of high conduction velocity.

### *The nature of the motor fibres to the muscle spindle*

Since the increase in the frequency of discharge of muscle spindle receptors in the present work results from contraction of intrafusal muscle fibres, the threshold at which activation of a particular spindle receptor occurred was taken to be identical with the threshold of the spindle motor fibres. Of twenty-one spindle receptors which were activated by stimulation of the muscle nerve, in only two was activation obtained below the alpha-maximum voltage. The effect occurred at the alpha-maximum voltage in three fibres, at just above the alpha-maximum voltage in three others (1·1; 1·1; 1·3). In thirteen fibres the threshold at which the spindle response could be driven was at, or more than twice, the alpha-maximum voltage.

An idea of the conduction velocity of spindle motor fibres was obtained by comparing these figures with those of Leksell (1945). Leksell determined the relative thresholds of gamma and alpha motor fibres (identified by measurement of conduction velocity) of hind-limb muscles of the cat. He found that the gamma fibres were activated after the stimulus strength was raised above the value which activated the least sensitive of the alpha motor fibres. Hence it seems that the two fibres (16 and 26), which were activated at 0·13 and 0·42 times the alpha-maximum voltage, were supplied by spindle motor fibres probably conducting in the alpha range of velocities: the receptors which were activated at stimulus strengths above

twice the alpha-maximum voltage were probably conducting at gamma velocity.

*Spontaneous discharge of mammalian muscle spindles*

Many spontaneously active receptors, which discharged when the muscle was slack, were encountered. The majority of these had receptor fibres of the slowly conducting type (Table 1). In nine animals under urethane anaesthesia only one fibre conducting impulses faster than 70 m/sec was spontaneously active. In contrast, twelve out of fourteen muscle spindles with fibres conducting impulses below 70 m/sec were spontaneously active. These spontaneous discharges were in all instances noted before the injection of succinylcholine or gallamine.

## DISCUSSION

The results indicate that both types of spindle receptors can be activated by repetitive stimulation of the muscle nerve (cf. Hunt, 1954; Harvey & Matthews, 1960). In addition, they indicate that although all spindle receptor fibres can probably be activated by repetitive stimulation of the muscle nerve, only a limited number also respond to single shocks to the muscle nerve, and these mostly lie in the upper range of conduction velocities. It seems, therefore, that the spindle receptors investigated in the present work fall into two groups. One group consists of receptors which are usually silent in slack muscle, possess receptor fibres of high conduction velocity and are activated by single shocks to the muscle nerve. They may also show an early discharge. The other group comprises receptors which are usually active spontaneously, are connected to receptor fibres of lower conduction velocity and are usually activated only by repetitive stimulation of the muscle nerve. They do not show an early discharge.

In many fibres, activation only occurred after complete or partial paralysis of the extrafusal muscle fibres with succinylcholine chloride. It is suggested that the effect obtained after succinylcholine is due to the absence of the unloading effect of extrafusal contraction when the intrafusal muscle fibres act unhindered on adequate stimulation of spindle motor fibres. One source of doubt as to the validity of this interpretation is the fact that succinylcholine causes marked excitation of the spindle receptors in addition to paralysing the extrafusal muscle fibres. It might be argued that succinylcholine, which blocks by depolarization, lowers the threshold of the receptors and pre-disposes them to repetitive discharges on stimulation of the muscle nerve. However, activation of a receptor has been obtained in the absence of the drug, the effects not differing qualitatively or quantitatively when repeated in its presence. Furthermore, the

effect has been obtained by blocking extrafusal contraction by the use of gallamine, which blocks the neuromuscular junction of skeletal muscle without exciting the spindle receptors. Gallamine does not block transmission across intrafusal end-plates after it has blocked extrafusal end-plates completely (Granit, Homma & Matthews, 1959).

Can the present findings be correlated with the histology of the muscle spindle? Considering first the intrafusal muscle fibres, it is suggested that the receptors which are activated by single muscle nerve shocks are influenced by a different type of intrafusal muscle from the receptors which are activated by tetanic stimulation alone. This suggestion is entertained because of evidence from three different sources, which indicates that both twitch and tonic intrafusal muscle fibres exist. First, Kuffler, Hunt & Quilliam (1951) recorded slow intrafusal potentials from the tenuissimus muscle of the cat. Secondly, Boyd (1958), using histological techniques, demonstrated the presence of two types of muscle fibres, large and small, in the muscle spindles of the cat's tenuissimus muscle. He later stated that the large intrafusal muscle fibres contracted in an all-or-nothing fashion, whereas the small intrafusal muscle fibres were capable of graded responses (Boyd, 1959). Thirdly, Eyzaguirre (1960) recorded propagated potentials from the muscle spindles of the cat's tenuissimus muscle. He maintained, on account of the absence of slow intrafusal potentials, that the intrafusal muscle fibres were twitch fibres. It is doubtful, however, if the recording of potential changes in this manner will exclude the existence of slow intrafusal muscle fibres, for Ginsborg has shown that slow muscle fibres of the chick are capable of propagated responses (Ginsborg, 1959).

Returning to the present results, the receptors of the first group, that is, those activated by single nerve shocks, possibly lie on twitch intrafusal muscle fibres, whereas the receptors in the second group (activated only by tetanic stimulation of the muscle nerve) lie on tonic intrafusal muscle fibres.

Whether these two groups of receptor fibres come from primary and secondary endings respectively cannot be answered definitely, because of lack of figures of the conduction velocities and fibre sizes of primary and secondary spindle receptor fibres from the gastrocnemius medialis and plantaris muscles of the rabbit. In the vastus muscle of the rabbit the primary and secondary receptor fibres measured 8–12$\mu$ and 6–9$\mu$ respectively inside the muscle (Barker, 1948). Hagbarth & Wohlfart (1952) found that the spindle receptor fibres measured 8–15$\mu$ but did not divide them into two groups, primary and secondary (cat's gastrocnemius medialis, soleus, and tibialis anterior muscles). Both these figures, which were obtained by direct measurement in fixed and stained preparations, are

unsuitable for comparing with the conduction velocities of the fibres studied in the present work. This is because the maximum expected conduction velocities from the studies in the two papers cited above would be 72 and 90 m/sec respectively (using a conversion factor of six) whereas fibres conducting at over 100 m/sec were encountered in the present work. Two other pieces of evidence, less direct than those quoted above, point to 70 m/sec as a reasonable figure which could be used in dividing spindle receptor fibres into primary and secondary. Hagbarth & Wohlfart (1952) showed that receptor fibres from certain hind-limb muscles of the cat occurred in three different diameter groupings, viz. 1–5$\mu$, 5–11$\mu$, 11$\mu$ and over (see also Lloyd & Chang, 1948). Secondly, Hunt (1954) found that spindle receptor fibres of the cat's gastrocnemius medialis and soleus muscles fell into two definite groups conducting at 24–72 m/sec and 72–120 m/sec respectively. A reasonable way of dividing the twenty-eight spindle receptor fibres studied in this work is to regard fibres conducting at 70 m/sec and above as coming from primary endings and those conducting impulses below 70 m/sec as originating from secondary endings.

This would imply that the great majority of primary endings lie on twitch intrafusal muscle fibres, since they can be activated by single shocks to the muscle nerve, and the majority of secondary endings on tonic intrafusal muscle fibres, needing a short tetanus for activation. However, the series is not large enough to say how many exceptions there may be to this postulate.

## SUMMARY

1. A study has been made of the responses of twenty-eight spindle receptor fibres from the gastrocnemius medialis and plantaris muscles of anaesthetized rabbits under conditions of maximal and minimal extrafusal contraction.

2. A technique by which intrafusal muscle fibres can be activated selectively is described. It consists of paralysing the extrafusal muscle fibres partially or completely by retrograde injection of succinylcholine, coupled with adequate stimulation of the muscle nerve by single shocks or brief tetani.

3. The spindle receptor fibres of high conduction velocity ($>$ 70 m/sec) were activated by single shocks to the muscle nerve. The majority of spindle receptor fibres of lower conduction (70 m/sec or less) were activated only by repetitive stimulation of the muscle nerves.

4. The voltage at which activation of the spindle receptors occurred was at or above the voltage which evoked a maximal twitch of the extrafusal muscle fibres. However, two endings were activated by stimuli which were only a fraction of the voltage which was needed to produce a maximal extrafusal twitch.

**5. An attempt is made to correlate the findings with the types of receptor ending and with the nature of intrafusal muscle fibres.**

I wish to express my gratitude to Mr J. E. Pascoe who suggested the problem to me and to the Medical Research Council for a grant to Mr J. E. Pascoe in connexion with this work.

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# DRINKING BY RATS DEPLETED OF BODY FLUID WITHOUT INCREASE IN OSMOTIC PRESSURE

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Strauss (1958) said that: 'Thirst may occur in a variety of situations in which there is reason to believe that the volume of body fluids is contracted without concomitant change in osmolality.' As Strauss implies, the evidence is only suggestive. The present experiments were carried out in order to determine whether reduction in effective fluid volume without increase in crystalloid osmotic pressure, by peritoneal dialysis or by bleeding, would cause rats to drink.

## METHODS

Albino rats of both sexes weighing between 150 and 500 g were used. During the experiments they were housed in individual metabolism cages with water available from a drinking meter (Fitzsimons, 1958) but no food. Urine was collected and measured by weighing, the bladder being emptied by suprapubic pressure at the start and finish of the experiment.

Effective fluid volume was reduced by peritoneal dialysis or by bleeding. Peritoneal dialysis was carried out in two ways; by intraperitoneal injection of hyperoncotic colloid dissolved in water or in 0·9% sodium chloride, or by placing Visking cellulose sacs containing hyperoncotic colloid dissolved in 0·9% sodium chloride in the peritoneal cavity. Gum acacia, polyvinyl pyrrolidone and polyethylene glycol in 25–50% (w/v) solutions were used. The Visking cellulose sacs were to prevent systemic absorption of colloid from the peritoneal cavity. The sacs were well tolerated, though the depletions produced were smaller than when the colloid was introduced directly in the peritoneal cavity. Animals were bled by cutting off the tip of the tail.

The experimental procedure was to inject a known weight of hyperoncotic solution, or insert a weighed Visking cellulose sac containing hyperoncotic solution through a small lumbar incision, or to bleed the animal a known amount, all procedures being carried out under ether anaesthesia. The rat was weighed to the nearest 0·1 g and placed in its metabolism cage, by which time it was recovering consciousness. Spontaneous drinking was recorded and urine collected for the next 6 hr. At the end of this time the rat was again anaesthetized and weighed and the change in weight of the rat was used as a measure of net fluid intake. The ascitic fluid or sac was recovered and weighed and the depletion produced in 6 hr obtained from the changes in weight.

Some experiments were made on nephrectomized rats by essentially the same procedure, except that there was no urine to collect. Bilateral nephrectomy was carried out through a lumbar incision, the colloid or sac introduced and the wounds in the muscle wall sealed to prevent escape of fluid, or the animal was bled.

The amount of water drunk, net fluid intake (i.e. change in body weight), depletion (i.e. increase in ascitic fluid or blood loss) and amount of urine were expressed as percentages of the initial body weight before the intraperitoneal procedures or bleeding.

In some experiments serum and dialysate freezing points were measured thermoelectrically, using a thermistor and simple bridge circuit. Blood samples were taken from the tail at the start and finish of the experiment and ascitic fluid was obtained at the same time as the final blood sample.

## RESULTS

### *The rate and magnitude of depletion*

The rate of accumulation of ascitic fluid in normal rats not allowed to drink after intraperitoneal injection of gum acacia dissolved in water is plotted in Fig. 1. Each point is the result from a different rat but all were given approximately the same dose of gum acacia per 100 g body weight.

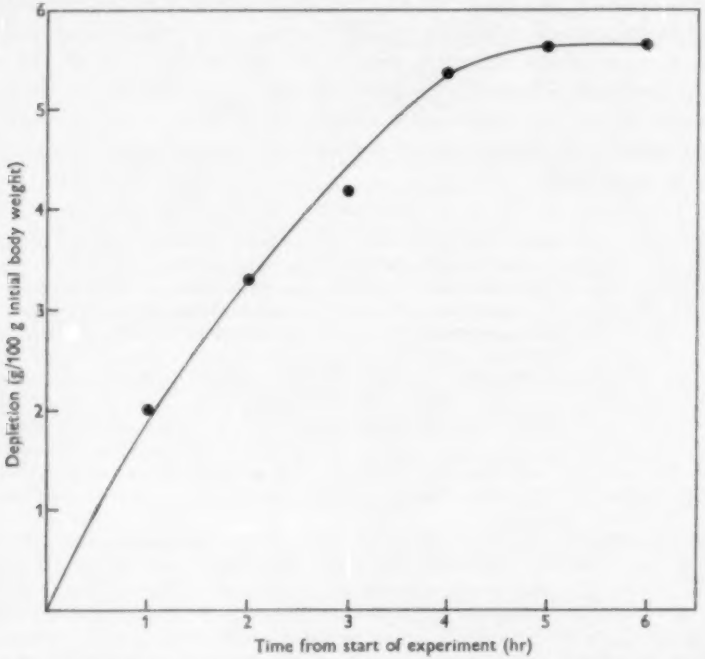



Fig. 1. The rate of accumulation of ascitic fluid in normal rats, not allowed to drink, after intraperitoneal injection of between 0·87 and 0·96 g of gum acacia/100 g body wt. in 50 % solution in water. Each point is the result from a single rat.

The time course of swelling of Visking cellulose sacs containing colloid dissolved in 0·9 % sodium chloride and suspended in 0·9 % sodium chloride is essentially the same.

The total depletions produced at the end of 6 hr by various doses of gum acacia are plotted in Fig. 2. Nephrectomy makes little difference to

the degree of depletion produced nor does it matter whether the colloid is dissolved in water or 0·9% sodium chloride, suggesting that the peritoneum is as freely permeable to Na and Cl as it is to water. Indeed, Na and Cl appear very rapidly in the peritoneal dialysate after colloid dissolved in water has been injected.

The few measurements of serum and dialysate freezing points in Table 1 are sufficient to show that there was no increase in total osmotic pressure after injection of colloid.

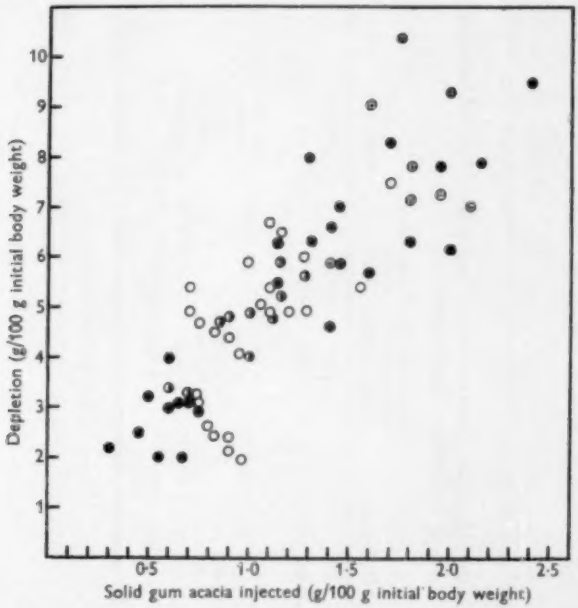



Fig. 2. Accumulation of ascitic fluid at 6 hr after various doses of gum acacia. Drinking was allowed. ○, Normal rats, gum acacia dissolved in water; ⊙, normal rats, gum acacia dissolved in 0·9% NaCl; ◑, nephrectomized rats, gum acacia dissolved in water; ●, nephrectomized rats, gum acacia dissolved in 0·9% NaCl.

### *The effect of body fluid depletion without increase in osmotic pressure on drinking and urine output of normal rats*

The effects of intraperitoneal hyperoncotic solutions and dialysing sacs on the amount of water drunk, net fluid intake and urine volume are given as mean values in Table 2 and the individual results are plotted in Fig. 3. Experimental animals generally drank more water and excreted less urine than the controls.

TABLE 1. Serum and dialysate freezing points, depletion and net fluid intake of normal rats after intraperitoneal injection of gum acacia

| Procedure | Initial serum freezing point (°C) | Final serum freezing point and time after initial serum freezing point: (°C) | (hr) | Dialysate freezing point (°C) | Depletion (g/100 g initial body wt.) | Net fluid intake (g/100 g initial body wt.) |
|---|---|---|---|---|---|---|
| Sham I.P. injection | −0·565 (3)* | −0·565 | 6 | — | — | −2·2 |
| Gum acacia (g/100 g body wt.) | | | | | | |
| 0·92 in 25 % soln. in $H_2O$ | −0·568 (3) | −0·504 | 6 | −0·526 | 3·6 | 0·9† |
| 0·91 in 50 %} | −0·596 (1) | −0·573 | 1 | −0·601 | 2·0 | −0·5 |
| 0·87 in 50 % soln. in $H_2O$ | −0·603 (1) | −0·557 | 2 | −0·582 | 3·3 | −0·3 |
| 0·96 in 50 %} | −0·591 (1) | −0·574 | 4 | −0·574 | 5·4 | −1·2 |
| 1·09 in 50 % soln. in 0·9 % NaCl | −0·576 (1) | −0·557 | 3 | −0·580 | 1·7 | −0·9 |
| Gum acacia in dialysing sac | −0·580 (4) | −0·570 | 6 | — | 3·0 | −1·6 |

* Mean values with the number of observations in parentheses. † Drinking allowed.

TABLE 2. The effect of depleting body fluid without increasing osmotic pressure on drinking and urine output of normal rats

| Procedure | Water drunk (ml./100 g initial body wt.) | Net fluid intake (g/100 g initial body wt.) | Depletion (g/100 g initial body wt.) range and mean† | Urine (g/100 g initial body wt.) |
|---|---|---|---|---|
| Sham I.P. injection | 0·46 ± 0·2 (10)* | −2·8 ± 0·26 | 0 | 1·85 ± 0·31 |
| Substance (g/100 g body wt.) | | | | |
| Gum acacia | | | | |
| 0·69–1·69 in 25 and 50 % soln. in $H_2O$ | 3·89 ± 0·56 (24) | 1·95 ± 0·45 | 1·96–7·55<br>*4·54* | 0·73 ± 0·16 |
| 1·42–2·1 in 50 % soln. in 9 % NaCl | 8·85 ± 0·6 (8) | 7·06 ± 0·31 | 5·87–10·45<br>*8·0* | 0·62 ± 0·19 |
| Polyethylene glycol | | | | |
| 0·35–0·82 in 50 % soln. in $H_2O$ | 3·18 ± 0·27 (10) | 2·1 ± 0·29 | 4·78–10·7<br>*7·3* | 0·14 ± 0·05 |
| Polyvinyl pyrrolidone | | | | |
| 0·47–0·86 in 50 % soln. in $H_2O$ | 3·85 ± 0·48 (10) | 1·61 ± 0·45 | 3·24–6·75<br>*4·9* | 0·63 ± 0·06 |
| Dialysing sac control | 1·45 ± 0·21 (25) | −2·39 ± 0·17 | 0 | 2·17 ± 0·18 (23) |
| Dialysing sac depletion | 2·28 ± 0·26 (30) | 0·2 ± 0·28 | 2·61–6·28<br>*4·33* | 0·71 ± 0·09 |

* Mean values ± S.E. of mean with the number of observations in parentheses. † Mean values in italics.

The rates of drinking in some depletion experiments and after intraperitoneal injection of 2 M sodium chloride are compared in Fig. 4. The animals injected with sodium chloride, which were treated in exactly the same way as the animals injected with colloid, started to drink as soon as they recovered from the anaesthetic and 30 min after the injection they had drunk appreciable quantities of water. On the other hand, none of the animals treated with colloid had started to drink at this time. Another difference was that urine volumes after hypertonic sodium chloride were

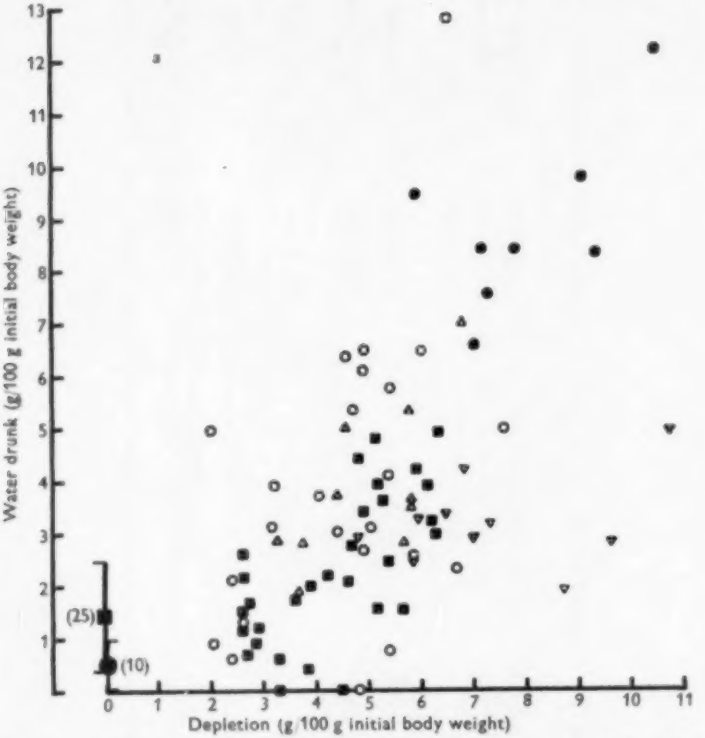
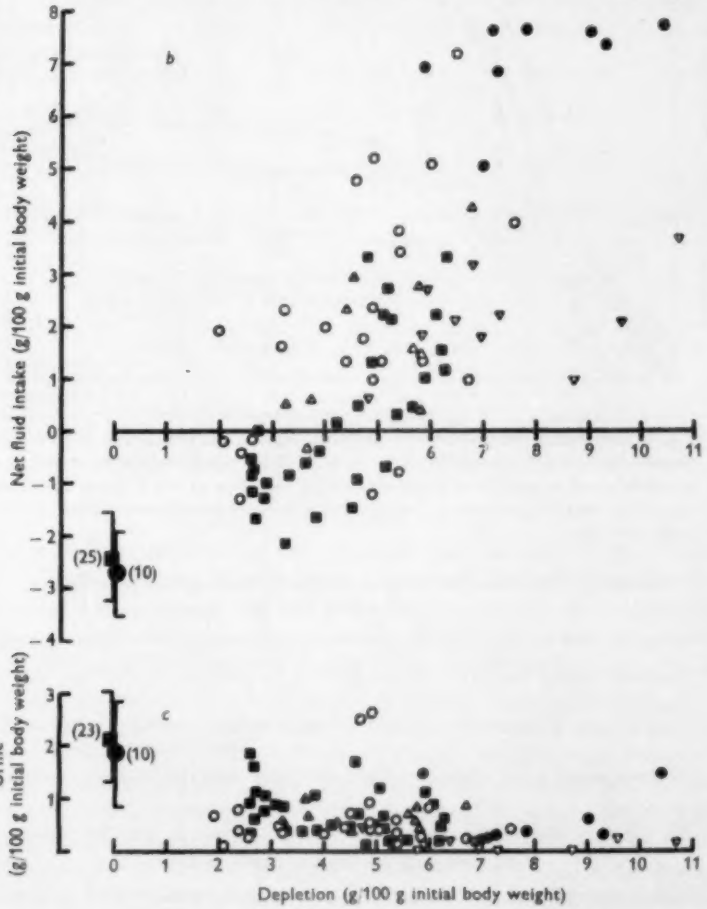



Fig. 3. (*a*) The amount of water drunk, (*b*) the net fluid intake and (*c*) the urine output of normal rats plotted against body fluid depletion. Each point is one observation, except the controls which are mean values ± S.D. with the number of observations in parentheses. ●, 1·4–2·1 g gum acacia/100 g body wt. in 50% solution in 0·9% NaCl and sham I.P. injection control; ○, 0·69–1·69 g gum acacia/100 g body wt. in 25 and 50% solution in water; △, 0·47–0·86 g polyvinyl pyrrolidone/100 g body wt. in 50% solution in water; ▽, 0·35–0·82 g polyethylene glycol /100 g body wt. in 50% solution in water; ■, dialysing sac depletion and dialysing sac control.

considerably greater than after doses of colloid which caused approximately the same amount of drinking (Fig. 4).

Another method of causing acute isotonic depletion of body fluid is to bleed the animal. The disadvantage of this method is that only small quantities of extracellular fluid can be removed without causing severe acute anaemia and haemorrhagic shock. Nevertheless, it was found that removal of rather more than $\frac{1}{3}$ the initial blood volume did not obviously alter the activity of the animals but did cause increased drinking (Table 3).




Figs. 3*b*, *c*. For legend see opposite page.

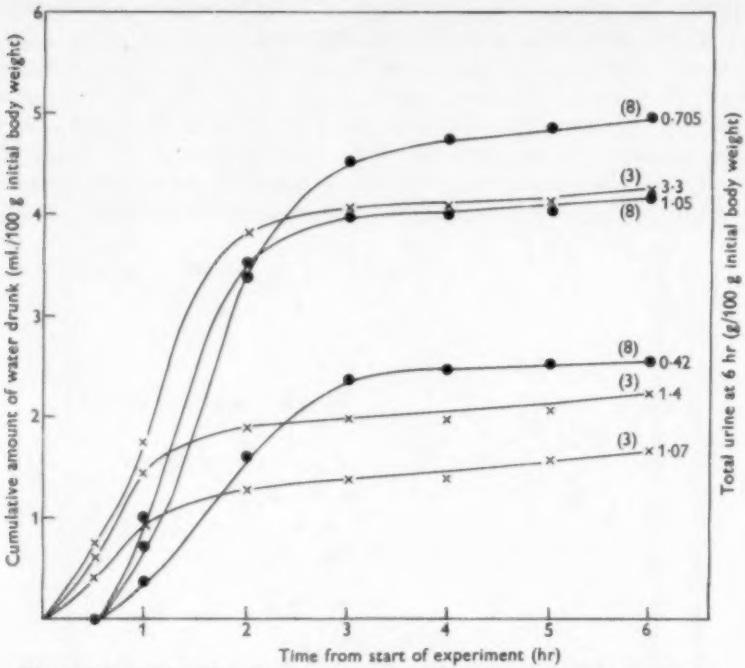

Cumulative amount of water drunk (ml./100 g initial body weight)
Total urine at 6 hr (g/100 g initial body weight)
Time from start of experiment (hr)
(8) 0·705
(3) 3·3
(8) 1·05
(8) 0·42
(3) 1·4
(3) 1·07

Fig. 4. Rates of drinking after intraperitoneal injection of gum acacia dissolved in water causing depletions of 2·7, 4·8 and 6·1 g/100 g initial body weight (●), and intraperitoneal injections of 2-M-NaCl causing increases of 3·4, 5·2 and 12·2% of the initial osmotic pressure (×); number of animals in parentheses. Urine output is on the right.

TABLE 3. The effect of acute haemorrhage on drinking and urine output of normal rats

| Procedure | Water drunk (ml./100 g initial body wt.) | Net fluid intake (g/100 g initial body wt.) | Amount of blood removed (g/100 g initial body wt.) | Urine (g/100 g initial body wt.) |
|---|---|---|---|---|
| Sham-bled | 0·59 ± 0·15 (5)* | −1·85 ± 0·16 | 0 | 0·81 ± 0·16 |
| Bled | 3·3 ± 0·58 (12) | −0·09 ± 0·33 | range 1·7–3·6 mean *2·81* | 1·72 ± 0·33 |

* Mean values ± S.E. of mean with the number of observations in parentheses.

### *The effect of body fluid depletion without increase in osmotic pressure on drinking by nephrectomized rats*

Similar experiments were carried out on nephrectomized rats to test whether drinking during the depletion experiments might be secondary to a renal response to the depletion. The results are given in Tables 4 and 5.

The amount of water drunk and net fluid intake of all nephrectomized animals after the various procedures except bleeding are plotted against depletion in Fig. 5. The results were the same as for normal animals: the treated nephrectomized animals drank more than the controls.

TABLE 4. The effect of depleting body fluid without increasing osmotic pressure on drinking by nephrectomized rats

| Procedure | Water drunk (ml./100 g initial body wt.) | Net fluid intake (g/100 g initial body wt.) | Depletion (g/100 g initial body wt.) range and mean† |
|---|---|---|---|
| Sham I.P. injection | 1·64 ± 0·26 (20)* | 0·33 ± 0·16 | 0 |
| Gum acacia (g/100 g body wt.) 0·59–1·27 in 50 % soln. in $H_2O$ | 4·71 ± 0·77 (10) | 3·5 ± 0·78 | 3·26–5·9 *4·7* |
| 0·3–2·4 in 50 % soln. in 0·9 % NaCl | 6·12 ± 0·53 (25) | 5·02 ± 0·5 | 1·99–9·5 *5·21* |
| Dialysing sac control | 1·81 ± 0·34 (12) | 0·51 ± 0·34 | 0 |
| Dialysing sac depletion | 3·38 ± 0·31 (16) | 2·27 ± 0·32 | 2·72–7·0 *3·96* |

* Mean values ± S.E. of mean with the number of observations in parentheses.

† Mean values in italics.

TABLE 5. The effect of acute haemorrhage on drinking by nephrectomized rats

| Procedure | Water drunk (ml./100 g initial body wt.) | Net fluid intake (g/100 g initial body wt.) | Amount of blood removed (g/100 g initial body wt.) |
|---|---|---|---|
| Sham-bled | 1·1 ± 0·27 (12)* | −0·23 ± 0·28 | 0 |
| Bled | 2·53 ± 0·36 (12) | 1·24 ± 0·34 | range 2·5–3·4 mean *2·97* |

* Mean values ± S.E. of mean with the number of observations in parentheses.

## DISCUSSION

There is little evidence in the literature that changes in extracellular fluid volume, or some function of extracellular fluid volume other than crystalloid concentration, can affect drinking directly. As recently as 1960 Holmes said: 'In the loss of isotonic fluids there is equal loss of water and salt and a shock-like syndrome results; in this situation there is no thirst.' Thirst accompanying diarrhoea is said to be well known (Strauss, 1958), but Strauss also refers to experiments (Nadal, Pedersen & Maddock, 1941) in which incipient circulatory collapse was produced in man by removal of fluid by intestinal aspiration, yet the subjects did not complain of thirst. The postprandial consumption of water by dogs (Gregersen, 1932) is also often attributed to secretion of isotonic digestive juice. Another frequently quoted example of isotonic fluid loss, in which there is said to be thirst, is haemorrhage. However, Holmes & Montgomery (1951, 1953) have reported that blood donors never complain of thirst. They also found that bleeding a dog of up to 40 % of its blood volume did not cause spontaneous drinking,

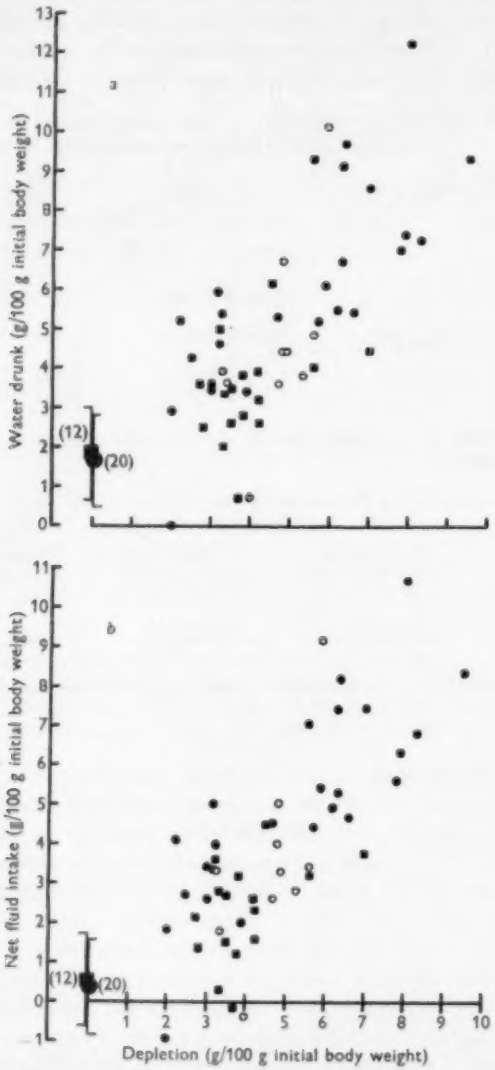



Fig. 5. (*a*) The amount of water drunk, and (*b*) the net fluid intake of nephrectomized rats plotted against body fluid depletion. Each point is one observation except the controls, which are mean values ± S.D. with the number of observations in parentheses. ●, Gum acacia, 0·3–2·4 g/100 g body wt. in 50% solution in 0·9% NaCl and sham I.P. injection control; ○, Gum acacia, 0·59–1·27 g/100 g body wt. in 50% solution in water; ■, dialysing sac depletion and dialysing sac control.

nor did it alter the amount of water drunk by the dog in response to hypertonic sodium chloride. They concluded that thirst only occurred if there were more complicated changes than simple blood depletion.

Salt depletion is a circumstance under which increased drinking of water is associated with a decrease in extracellular fluid volume. McCance (1936) studied the effects of sodium depletion in three healthy human subjects. As sodium depletion progressed, two subjects complained of thirst, though one sometimes called it a funny sensation, and drank large quantities of water without relief. The third subject experienced a sensation described as an aberration or lack of taste and which was quite different from true thirst. Though the fall in extracellular fluid volume which occurs *pari passu* with the loss of sodium could be the drinking stimulus, the low sodium concentration itself may stimulate a rather ill defined salt drive or appetite which is sometimes mistakenly interpreted as need for water or thirst. The dramatic relief afforded by sodium chloride at the end of the experiment, which occurred before water was drunk, makes this explanation plausible.

Sodium depletion in animals has also been shown to cause polydipsia. Holmes & Cizek (1951) found that dogs depleted of extracellular electrolyte by sucrose diuresis and thereafter maintained on a low salt diet started to drink increasing quantities of water. In other experiments Cizek, Semple, Huang & Gregersen (1951) showed that depleting dogs of extracellular electrolyte by peritoneal dialysis with 5% glucose caused polydipsia after a period of depression lasting a day or so. At first there was no shrinkage of volume, but later extracellular space and plasma volume decreased by 10–20%, while voluntary water intake doubled even though the extracellular fluid was hypotonic and the intracellular fluid permanently expanded. Similar results were obtained on rabbits (Huang, 1955). In an earlier study on dietary sodium deficiency in rats, Swanson, Timson & Frazier (1935) found polydipsia and polyuria, and this has been confirmed by Radford (1959). More acute depletion by peritoneal dialysis gave variable results (Semple, 1952) and subsequent water intake depended on food intake. Holmes (1960) has recently summarized the results of salt depletion experiments, saying that thirst does not occur in the acute phase of hyponatraemia, but polydipsia may develop in the chronic phase.

In the present experiments drinking occurred when an acute alteration in the distribution of body fluid was produced without increasing the crystalloid osmotic pressure. It seems that an acute fall in effective volume will cause drinking within an hour or so, without the delay of a day or more observed in salt-depletion experiments. Normal or nephrectomized animals, depleted by various colloids dissolved in 0·9% sodium chloride or water, or depleted by dialysing sacs, or by bleeding, drank more than

the controls. Table 1 shows that there was no increase in osmotic pressure, which is the well established stimulus to drinking (Fitzsimons, 1961), and indeed the time course of drinking and urine output were not the same as after intraperitoneal injection of sodium chloride (Fig. 4). The sac experiments make it unlikely that drinking was due to a systemic effect of the colloid, although they do not entirely exclude the possible systemic effects of smaller molecules which pass through the sacs. This of course would not explain the effects of bleeding. The fact that nephrectomized animals drank when they were depleted rules out the possibility that drinking was secondary to a renal response to the depletion.

The mechanism of the drinking produced by isotonic depletion of body fluids is quite obscure. It may be related to salt appetite. McCance's (1936) experiments suggest that the sensation aroused by salt deprivation is different from thirst. His results did not show whether the low serum sodium concentration or the accompanying fall in extracellular fluid volume was the drinking stimulus, though the immediate relief afforded by sodium chloride suggests the former. On the other hand, Denton & Sabine (1961) have shown that a sharp fall in plasma volume induced by sodium deficiency caused sheep to drink solutions of sodium salts in preference to water or potassium chloride solution, and that the preference did not depend on the plasma sodium concentration. Whether the sensation experienced by the rats in the present experiments is the same is impossible to determine; the important fact is that it leads to drinking of water.

SUMMARY

1. Haemorrhage or peritoneal dialysis caused both normal and nephrectomized rats to drink more water than the controls.

2. This is ascribed to a sensation akin to thirst produced by diminution in volume of the body fluids without increase in osmotic pressure.

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# TENSION CHANGES IN CRAYFISH STRETCH RECEPTORS

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Although much is known about various stretch receptors no information seems to be available on their mechanical properties; yet these properties are often considered to be responsible for certain characteristics of the nervous response of the receptor to stretch (e.g. Matthews, 1931, 1933; Loewenstein, 1956; Finlayson & Lowenstein, 1958; Lippold, Nicholls & Redfearn, 1960; and many others). It therefore seemed of some interest to examine the stretch–tension relation of crayfish stretch receptors, which can be isolated and whose behaviour in many respects has already been analysed in great detail (Wiersma, Furshpan & Florey, 1953; Kuffler, 1954; Eyzaguirre & Kuffler, 1955; Florey, 1956). A brief report of our results has already appeared (Krnjević & van Gelder, 1960).

## METHODS

All the stretch receptor organs were taken from specimens of *Astacus fluviatilis* (sub-variety *torrentium*). During the dissection the preparation was kept submerged in crayfish saline, and after isolation the receptor organs were transferred in a platinum spoon, together with a small volume of fluid, to the recording chamber. Inside the glass-bottomed Perspex chamber the receptors remained floating in saline (0·5–1 ml.) throughout the experiment. The saline was derived from van Harreveld's (1936) well-known solution, and had the following composition (mM): $Na^+$ 202, $K^+$ 5·0, $Ca^{2+}$ 9·4, $Mg^{2+}$ 2·3; $Cl^-$ 220, and Tris acid maleate buffer 10·5; with a pH of 7·1–7·2. In some experiments 4·7 mM L-monosodium glutamate was also present.

*Tension measurements.* One end of the receptor bundles was held by the closed tips of a watch-maker's fine forceps. The other end of one or both receptor organs was tied with 50 $\mu$ silver wire to a fine glass hook attached to the movable anode of an RCA 5734 mechano-electric transducer. The forceps and the transducer were both mounted on a horizontal threaded shaft, and they could be separated in a predictable manner by rotating this shaft (Fig. 1).

The stretch receptors are quite small; the slow-adapting organ, in particular, can have a diameter of 50 $\mu$. Their mechanical properties are apparently comparable with those of muscle in general, so that the total tension developed during stretch is in the order of only a few milligrams (cf. Kuffler, 1954). As the RCA 5734 transducers are very sensitive to

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changes in temperature and in voltage supply, two were used differentially to minimize drift caused by these factors.

The natural frequency of the transducer with the glass hook attached was in the order of 100 c/s, more than adequate to deal with the relatively slow changes of tension produced in these experiments. To calibrate recorded tensions accurately known weights were suspended from the glass hook of the transducer held at the appropriate angle. The output was linear over the relevant range of tensions. The compliance of the system was negligible; to displace the hook by 100 $\mu$ required a force of 200 mg.

*Nerve impulses.* The saline in the recording chamber was covered with a layer of liquid paraffin. The saline was earthed through an Ag-AgCl electrode covered with gauze or agar-agar. Afferent impulses from the nerve were recorded either in a monopolar fashion with one 50 $\mu$ silver wire on the nerve in the paraffin, or differentially with two such leads.

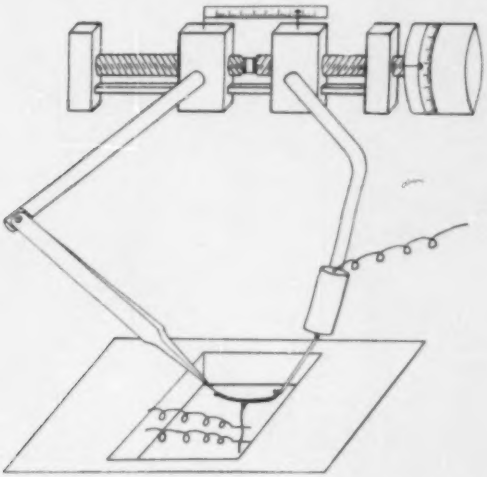

Fig. 1. Diagram of experimental set-up. One end of receptor organ is tied to fine glass hook connected to movable anode of transducer valve. Recording silver leads are in contact with nerve branch containing axon of receptor neurone.

*Procedure*

In most cases, in order to reduce handling, the two receptor organs (fast- and slow-adapting) were first mounted together. When initial observations had been completed, the thicker bundle of the fast-adapting receptor was disconnected on one side or cut from the forceps and observations were repeated while stretching only the thin bundle. In several cases one of the two organs (usually the thicker) was left untied from the beginning, so that only one exerted any appreciable tension. The two receptors are in very close relation to one another and so cannot be freed completely without great risk of damaging one or both. As has already been shown by Wiersma *et al.* (1953), even after cutting a bundle the corresponding receptor may be activated if the remaining bundle is stretched sufficiently (see below). Useful observations were made on sixteen isolated preparations, kept at room temperature (18–22° C).

## RESULTS

### *Changes in tension caused by stretching*

When a receptor organ is gradually pulled out, the tension initially rises only very slowly. Zero length was therefore a somewhat arbitrary length, determined partly by inspection with the microscope (showing that the resting folds had straightened out) and partly by evidence that the tension was beginning to increase. Resting length *in situ* was not used for comparison, since the length *in situ* varies considerably with the degree of

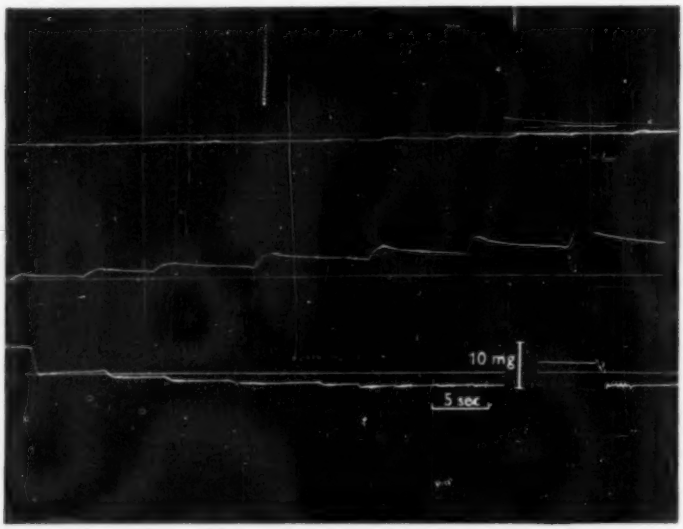


Fig. 2. Tension changes produced by stretching slow-adapting receptor organ in steps of 0·1 mm up to a maximum of 1·8 mm; in bottom row, length was reduced in steps of 0·2 mm. Zero length was 2·7 mm. Sequence to the right of calibration lines shows abolition of tension when moderately stretched receptor was cut with scissors at end of experiment.

abdominal flexion; in any case, the thinner bundle is not straight *in situ*, being attached at several points. In practice, the zero length was within the range of 1–3 mm.

In Fig. 2 a complete sequence of tension changes is illustrated. The bundle was stretched in steps of 0·10 mm, up to a maximum extension of 1·8 mm (i.e. an increase of 2/3 over the zero length). Several features are evident which were characteristic of all our observations. The tension did not increase linearly, but more and more steeply. At each increment in length there was a tendency for the tension to reach a peak that was not

maintained: this was seen especially clearly when the stretching exceeded 1 mm. The tension fell away from its peak value at first rapidly and then more slowly.

On reducing the length of the stretched bundle the tension falls suddenly, although it may creep up again somewhat before the next step down, as is shown in Figs. 2, 5 and 9. This phenomenon of a creeping up of tension was always much less evident than the corresponding creeping down during stretching.

The tensions measured in these experiments were so small that appreciable changes in tension were sometimes produced with no tissue attached, presumably by the surface tension of films of water between the forceps and the glass hook. For this reason controls were performed regularly, such as the demonstration at the end of the experiment that cutting the bundle abolished the tension and then repeating sequences of stretch and relaxation. A control cut is shown at the end of Fig. 2. It is clear that the tension produced by a moderate stretch of the same bundle disappeared when the bundle was cut. Although an appreciable amount of tension could sometimes be produced spuriously, at its greatest this amounted to only 14 % of the tension developed by stretching a bundle in the same experiment.

From traces like those in Fig. 2 plots were made of tension against stretch. In fifteen cases the plots gave rather similar curves, concave upwards and showing nearly always a distinct though variable hysteresis (e.g. Figs. 4, 7 and 10). There was no qualitative difference between observations made while stretching both receptors simultaneously or either independently. For instance, in Fig. 3 curves are shown of the tension changes caused by first stretching both bundles (open circles) and then stretching the thin bundle only (after cutting the thicker one). The thicker bundle is much larger than the thin one but its fibrils are usually folded rather loosely, so that although it is much thicker it seldom contributed as much as 50 % to the total tension when both were pulled together.

It is clear that the receptors did not obey Hooke's law. They behaved, in fact, very much like many other tissues (e.g. Stacy, 1957) and like vertebrate muscle fibres in particular (Ramsey & Street, 1940; Buchthal, 1942; Buchthal & Rosenfalck, 1957).

The receptor organs could be stretched by about 100 % of the initial length without any obvious damage, although inspection of curves suggested that there was some irreversible loss of resistance to extension if the length was increased by much more than 50 %, as in the case of muscle fibres (Buchthal, 1942).

The extent to which a dynamic phase (overshoot) of tension was observed depended on the rate of stretching. At the fastest rate of stretching that could be managed conveniently (0·4 mm/sec) the peak tensions exceed the plateau very substantially. Hence, if a series of such quick stretches is compared with a sequence of very slow stretching (at 0·01 mm/sec) a

marked excess of tension is immediately evident (Fig. 4, *A* and *B*). It can also be seen that the very slow stretching prevented the appearance of any pronounced hysteresis (in Fig. 4*B*).

Although the amount of overshoot of tension during the dynamic phase was related to the rate of stretching, for a given rate of stretching the overshoot was an approximately constant fraction of the final increment of plateau tension (Table 1). Hence, in general, the greater the increase in tension, the greater was the corresponding overshoot.

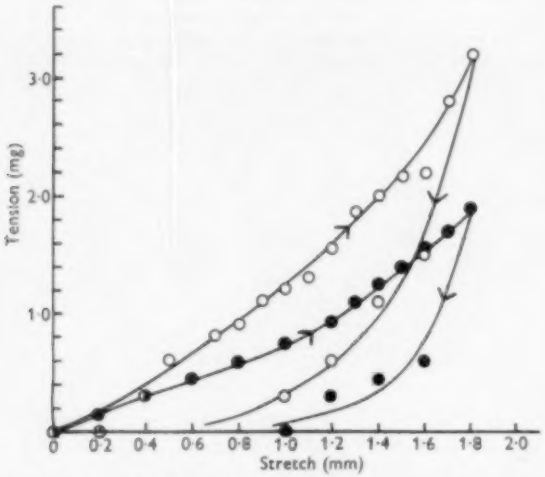


Fig. 3. Stretch–tension relation of (*a*) fast and slow-adapting receptor organs together (○—○), and (*b*) slow-adapting receptor organ after cutting fast-adapting one (●—●).

The tensions developed by various receptors at a given stretch differed considerably, owing at least partly to differences in zero length and variations in the thickness of individual organs. This could not be assessed with accuracy, since the receptors did not have a constant thickness and shape at different points along their length. The tension developed by a 1·0 mm stretch varied by a factor of nearly 30 in a series of twelve different trials with slow-adapting receptors: the mean tension was 1·8 mg, with a range of 0·3–8·5 mg. Four trials with fast-adapting receptors gave a mean tension of 1·1 mg (range 0·8–1·5 mg) at a stretch of 1·0 mm.

There was somewhat less variation between the rates of change of tension ($dF$) with increasing stretch ($dl$) in different preparations. In Table 2 are given representative values of $dF/dl$ at 4 degrees of extension, for three slow-adapting and two fast-adapting receptors.

As the receptors did not obey Hooke's law, it is not possible to calculate an elastic modulus that will describe the elastic properties over more than a limited range. Nevertheless, it may be of some interest to compare the elastic modulus in a given range with values obtained for ordinary muscle. The principal source of inaccuracy is due to some uncertainty about the cross-sectional area. In the present calculations it is assumed that the receptors were circular in cross-section. Five slow-adapting receptors, measured as accurately as possible with a magnification of 80 times, had diameters within the range 30–50 $\mu$. Values of the elastic modulus ($E$) were

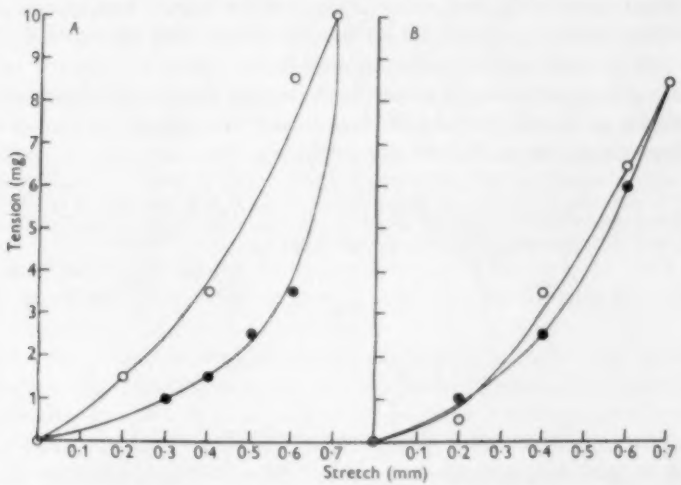


Fig. 4. Stretch–tension relation of slow-adapting receptor organ when stretched in steps, *A*, at relatively quick rate of 0·4 mm/sec, and *B* at slow rate of 0·01 mm/sec.

TABLE 1. Ratios of maximal change in tension (measured at peak) to final change (measured during plateau at a fixed time after peak). Receptor organs were all stretched in steps at approximately same rate (about 0·1 mm/sec). In each column values are in order of increasing stretch. Note that *A*, *E* and *F* were slow-adapting receptors, *B*, *C* and *D* fast-adapting

| | *A* | *B* | *C* | *D* | *E* | *F* |
|---|---|---|---|---|---|---|
| | 1·5 | 1·0 | 3·0 | 2·0 | 2·0 | 2·0 |
| | 1·25 | 1·2 | 4·0 | 1·5 | 3·0 | 1·5 |
| | 1·4 | 1·8 | 1·6 | 3·0 | 2·0 | 1·5 |
| | 1·5 | 2·1 | 2·0 | 2·0 | 2·0 | 2·0 |
| | — | 1·5 | 4·0 | 2·0 | 2·0 | 2·0 |
| | — | 1·6 | 2·2 | 2·0 | 1·5 | 1·8 |
| | — | — | 3·0 | 2·0 | 1·2 | 2·5 |
| | — | — | — | 1·7 | 1·8 | 2·3 |
| | — | — | 2·8 | — | — | — |
| Mean | 1·4 | 1·5 | 2·8 | 2·0 | 1·9 | 1·9 |

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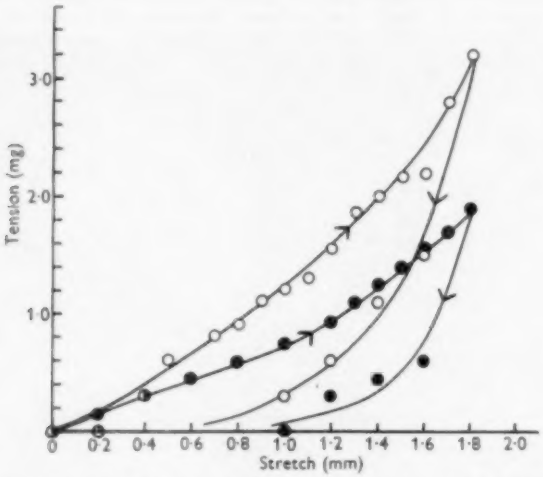


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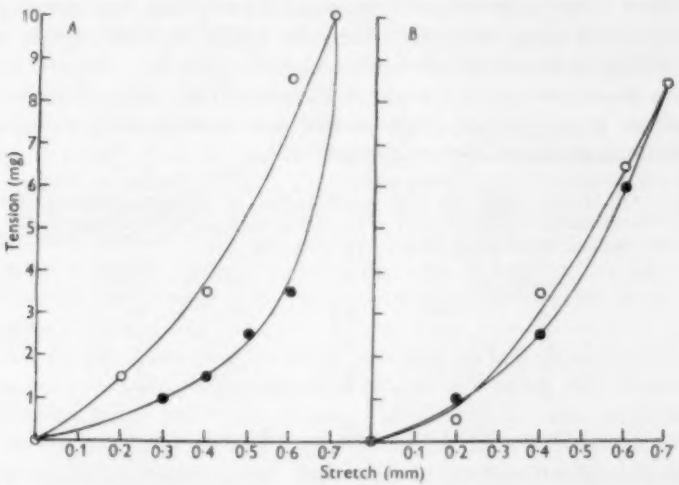


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| | *A* | *B* | *C* | *D* | *E* | *F* |
|---|---|---|---|---|---|---|
| | 1·5 | 1·9 | 3·0 | 2·0 | 2·0 | 2·0 |
| | 1·25 | 1·2 | 4·0 | 1·5 | 3·0 | 1·5 |
| | 1·4 | 1·8 | 1·6 | 3·0 | 2·0 | 1·5 |
| | 1·5 | 2·1 | 2·0 | 2·0 | 2·0 | 2·0 |
| | — | 1·5 | 4·0 | 2·0 | 2·0 | 2·0 |
| | — | 1·6 | 2·2 | 2·0 | 1·5 | 1·8 |
| | — | — | 3·0 | 2·0 | 1·2 | 2·5 |
| | — | — | — | 1·7 | 1·8 | 2·3 |
| | — | — | 2·8 | — | — | — |
| Mean | 1·4 | 1·5 | 2·8 | 2·0 | 1·9 | 1·9 |

thus calculated for the three slow-adapting receptors *A*, *B* and *C* (cf. Table 2), by means of the given values of $dF/dl$ for a stretch of 0·5 mm and 1·5 mm respectively, and equation 1:

$$E = \frac{dF}{dl}\frac{l}{\pi r^2}, \tag{1}$$

where $l$ is the total length, and $r$ the bundle radius at zero length corrected to allow for a reduction in cross-sectional area during stretch (assuming that the bundle volume remains constant). The calculated values of $E$ at 0·5 mm (i.e. in the region 120–130 % of zero length) are $0{\cdot}7–7{\cdot}0 \times 10^6$ dyn/cm². They agree fairly well with values of $E$ for resting frog muscle given in the literature (e.g. $0{\cdot}98 \times 10^6$ (Wöhlisch, 1932); $2{\cdot}5 \times 10^6$ (Sichel, 1934); $0{\cdot}5 \times 10^6$ (Buchthal, 1942), all in dyn/cm²).

For a greater stretch of 1·5 mm (in the region 170–180 % of zero length) the slopes of $dF/dl$ were much steeper and the respective values of $E$ correspondingly larger, 7·6–57 dyn/cm².

TABLE 2. Changes of tension per unit stretch ($dF/dl$) at different degrees of extension: values were calculated from tangents to curves such as those in Figs. 3, 4, 5 and 10. Receptors *A*, *B* and *C* were slow-adapting, *D* and *E* fast-adapting

| $l$ (mm) | 0·5 | 1·0 | 1·5 | 2·0 |
|---|---|---|---|---|
| *A* | 0·35 | 0·6 | 2·0 | — |
| *B* | 3·5 | 5·5 | 8·0 | — |
| *C* | 3·5 | 7·0 | 15 | 28 |
| *D* | 1·0 | 1·5 | 2·5 | 7·0 |
| *E* | 1·5 | 2·5 | 6·0 | 45 |

*Properties of slow- and fast-adapting receptors*

The data already presented show that there is little qualitative or even quantitative difference between the thin (slow-adapting) and thick (fast-adapting) receptor bundles, at least in so far as their more obvious mechanical properties are concerned. The curves of tension against stretch are much the same (compare Figs. 3 and 10); and values of the tension (see above) and of $dF/dl$ at different degrees of extension (Table 2) are well within the same range for the other type of receptor. However, since the fast-adapting receptors are much thicker, it follows that they must have a much lower coefficient of elasticity. In fact, the values of $E$ calculated as above for the two fast-adapting receptors in Table 2 (*D*, *E*) are 0·14 and $0{\cdot}21 \times 10^6$ dyn/cm² at a stretch of 0·5 mm, and 0·68 and $1{\cdot}6 \times 10^6$ dyn/cm² at a stretch of 1·5 mm respectively. These values are consistently less than the corresponding values of $E$ for the slow-adapting receptors already given.

One other feature should perhaps be mentioned. The dynamic phases of tension change were sometimes more pronounced when stretching the

thicker bundles than the thinner. In other words, the tension tended to fall off from the peak to a greater relative extent. This is noticeable in some of the figures given in Table 1. However, as can also be seen in Table 1, there was a good deal of overlap between the two groups, and it is not at all certain that this is a systematic difference.

*Relation between stretch and nerve discharge*

It is of some interest to know whether the observed acceleration in the discharge is related to the change in length or to the change in tension.

*Slow-adapting receptors.* Parts of a typical sequence of changes in rate of firing and tension during stretching in steps are shown in Fig. 5. Individual steps during stretch or release can also be seen enlarged in Fig. 6. Examination reveals a certain correspondence between the discharge and the tension, both showing at each step a dynamic phase which was not maintained. If we plot values of tension and of the firing frequency (during the relatively steady plateaus) against stretch, curves are obtained similar to those in the lower half of Fig. 7. They show that, like the tension, the firing rate increases progressively faster with increasing stretch. The general resemblance between such curves suggests that the tension and the firing rate may be simply related to each other. This is found to be the case if firing rate is plotted against the measured tension for the same receptor, as in the upper half of Fig. 7.

When the receptors were stretched very slowly, there was no overshoot of tension and the firing rate also had no dynamic phase, but there was a slow decline in the frequency of firing over several minutes; thus the receptors as a rule do show some slow-adaptation. Measurements of plateau tension and of the corresponding firing rate at different lengths during release also fell about the same straight line usually, except when the fully stretched state was maintained for several minutes, since the frequency of discharge declined more quickly than the usual creeping down of tension.

The slopes of firing against tension varied considerably in different experiments. The full range of ten estimates was 2–35 impulses/sec/mg. Receptors with a low elastic modulus tended to give a relatively steep relation between firing and tension. For instance, receptor *A* from Table 2, with low values of $dF/dl$, increased its rate of firing by 13 impulses/sec for every milligram increase in tension, whereas receptor *C*, with high values of $dF/dl$, accelerated by only 2·5 impulses/sec/mg.

The receptor whose behaviour is illustrated in Fig. 8 was an interesting example of the fact that, even after cutting a slow-adapting receptor bundle, its neurone may be caused to fire by stretching sufficiently the remaining fast-adapting receptor bundle (because of interconnecting

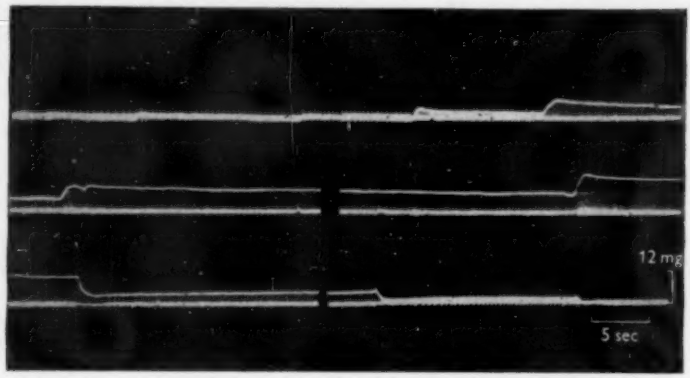


Fig. 5. Impulses in slow-adapting receptor axon and tension exerted by same receptor organ while being stretched in steps of 0·1 mm up to a maximum of 2·2 mm. Length was then reduced in steps of 0·1 mm. Only parts of complete sequence are illustrated. Zero length was 2·1 mm. Spikes had an amplitude of 200 $\mu$V.

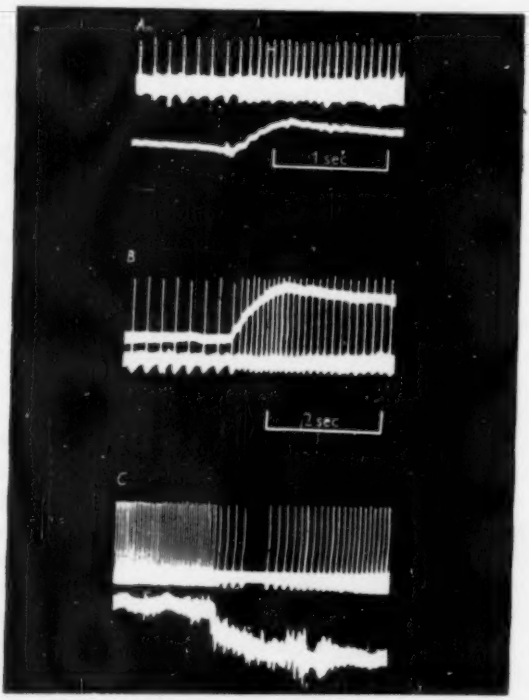


Fig. 6. Magnified traces showing tension and impulses, in *A* and *B* during stretch of 0·1 mm, in *C* during release by 0·1 mm. Traces have been retouched.

strands of tissue in the region of the neurones, as shown by Wiersma *et al.* 1953). However, this is evidently an inefficient method of activating the neurone; hence the very moderate inclination of the lower straight line in Fig. 8 corresponding to a change in frequency of only 0·25 impulse/sec/mg, i.e. only 1/10th of the lowest value observed in other receptors. Presumably not more than 1/10th of the tension developed in the thicker bundle is transmitted to the slow-adapting neurone via the connecting strands.

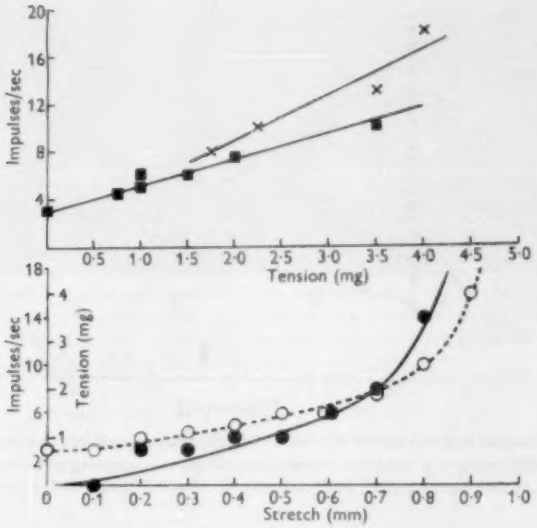


Fig. 7. Above: ■, relation between discharge of receptor and tension during plateau of tension after each increment in length; ×, corresponding relation for peak values of dynamic phase of tension. Below: ●, relation between tension and stretch of slow-adapting receptor organ; ○, relation between receptor discharge and stretch.

It therefore seems that over short periods of time the rate of discharge of the slow-adapting receptor may be determined principally by the tension in the receptor organ. The correspondence in time between the dynamic phases of tension and firing might suggest that the same simple relation holds throughout. But if one plots the peak firing rate against the peak tension one finds that, though the two variables are again related linearly, in the majority of cases the slope is substantially steeper (cf. the two sets of straight lines in Figs. 7 and 9).

*Fast-adapting receptors.* A typical sequence of changes in firing and tension while stretching in steps of 0·1 mm is shown in Fig. 9. As already

stated, the tension changes were not grossly different from those seen with slow-adapting receptors (see also Fig. 10). But the discharge adapted much more rapidly, so that it usually ceased altogether within a number of seconds. On the other hand, if the peak rate of discharge was plotted against the peak tension a linear relation was again found (Fig. 10); in three experiments the slopes had values of 3–5 impulses/sec/mg, near the lower end of the observed range for slow-adapting receptors.

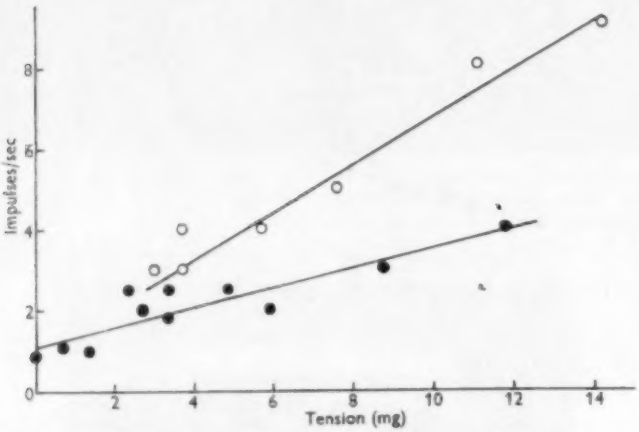


Fig. 8. Changes in firing rate of slow-adapting receptor caused by tension changes in adjacent fast-adapting receptor, transmitted via interconnecting strands. ●, values obtained during plateau after each stretch; ○, values at peak of dynamic phase of tension.

## DISCUSSION

It is not surprising that the stretch receptors should have mechanical properties very similar to those of muscle, since somewhat modified muscle makes up the bulk of the receptor bundle (Alexandrowicz, 1951; Florey & Florey, 1955). Like muscle, therefore, the receptors behave in a manner characteristic of bodies with both damped and undamped elements. In this respect the slow- and fast-adapting receptors are qualitatively very similar. However, unlike the slow-adapting receptors, the fast-adapting organs have a substantially smaller resistance to stretching, with a smaller elastic modulus than is common for muscle.

From equation (1) it can be seen that the change in length needed to produce a given change in tension per unit area of cross-section is proportional to $l/E$. The length of the fast-adapting receptor *in situ* is about 20% greater than that of the slow-adapting receptor. If one takes a representative figure of 1/10 for its elastic modulus, the fast-adapting receptor

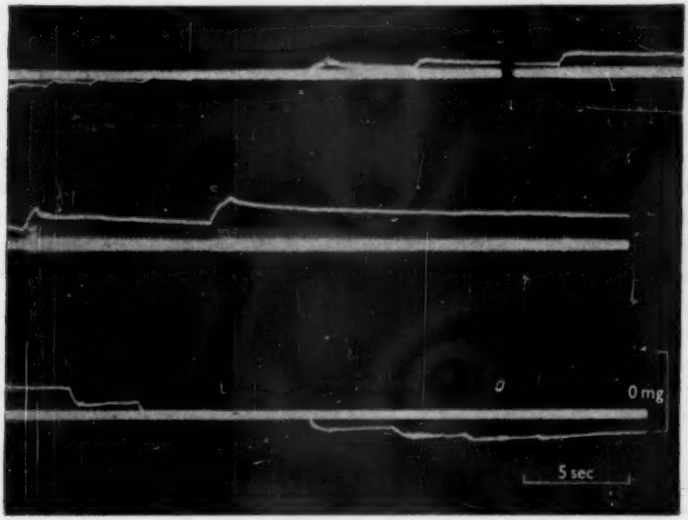


**Fig. 9.** Traces showing changes in firing and tension during stretch of a fast-adapting receptor organ in steps of 0·1 mm up to a maximum of 2·3 mm. Length was then reduced in three steps of 0·1 mm and four further steps of 0·5 mm. Zero length was 2·0 mm. Spikes had an amplitude of 120 $\mu$V.

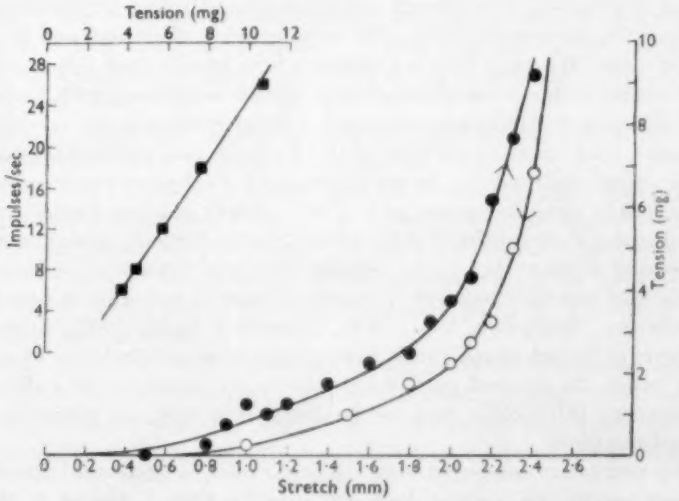


**Fig. 10.** Closed and open circles indicate tension exerted by fast-adapting receptor organ during stretch and relaxation in steps. Straight line through squares gives relation between discharge of receptor and tension at peak of dynamic phase.

would require an elongation twelve times greater to produce a given change in tension per unit area. The great difference between the amounts of extension needed to excite the two types of receptors *in situ* (cf. Wiersma *et al.* 1953; Florey & Florey, 1955) may be caused largely by their different mechanical properties. Some differences in neuronal characteristics, such as the electrical threshold (cf. Eyzaguirre & Kuffler, 1955) or the orientation of dendrites (Florey & Florey, 1955) may, of course, also play a part.

The different rates of adaptation of the fast and slow receptors cannot easily be ascribed to their mechanical properties: the slight tendency of the tension of fast-adapting receptors to fall more rapidly does not seem either sufficiently pronounced or regular in its occurrence (Table 1) to account entirely for the consistently quicker rate of adaptation. In other experiments (unpublished observations) we have found that the rates of adaptation of individual receptors to d.c. polarization are rather similar to the rates of adaptation observed after stretching. Moreover, we have also found that under the influence of certain drugs (which do not alter the mechanical properties) fast-adapting receptors may become slow-adapting. It seems that the principal factor in determining the rates of adaptation of the two kinds of receptors must be the electrical properties of the respective neurones.

*Relation between tension and firing*

Our observations have shown remarkably consistently a linear relation between the tension exerted by the receptor when stretched, and the rate of discharge. We have here a receptor which clearly does not provide a logarithmic-to-linear transformation of signals in keeping with Fechner's law. (If firing is plotted against length, a linear relation might conceivably be found between the logarithm of the *discharge* rate and the length, but certainly not the reverse.) As stretch-receptor mechanisms have proved in general to be remarkably similar in a wide variety of living forms, it would be surprising if they differed very radically in this respect. One cannot help wondering whether the linear relation observed between the logarithm of the load and the discharge frequency of stretch receptors in vertebrate muscle (e.g. Matthews, 1931, 1933; Fessard & Sand, 1937) is really a property of the actual receptors; when a large muscle is stretched by a given load, much the greatest part of the tension is exerted by the bulk of the muscle, and this tension may not be simply related to the tension in individual receptors.

Our results are consistent with the linear relation observed between cat muscle length and spindle discharge rate by Eldred, Granit & Merton (1953). In their experiments the muscle was extended by not more than 25% of its resting length. The authors point out that within this range an

indistinguishable result would be achieved if spindle frequency were proportional to the logarithm of the length. The converse is also true: within this range the results would probably be similar if the logarithm of the discharge were proportional to the length. For instance, in Fig. 7 the relation between extension and firing rate is approximately linear up to 0·7 mm (this is equivalent to a 30 % change in length). The lack of linearity between firing rate and extension is shown clearly in the experiments of Eldred *et al.* (1953) after de-afferentation (in their Figs. 2 and 3), when the spindle was allowed to discharge over a much greater range of frequency.

According to Florey & Florey (1955) the dendrites of the receptor neurones ramify extensively within the central region of the receptor organ over a length which may be as much as 0·5 mm. Since the muscular component does not extend continuously through the central part of the receptor (Alexandrowicz, 1958) the load on the dendrites cannot be reduced by parallel contraction of the muscle. Kuffler (1954) thus found that excitation of the efferent nerves to the muscle did not produce a silent period in the afferent discharge. Contraction of the muscle always caused an acceleration of neuronal firing, and he concluded that the dendrites were effectively in series with the muscular component. Changes in bundle tension, therefore, should be transmitted directly to the dendrites, and in the steady state the load on the dendrites should bear a simple relation to the tension exerted by the whole receptor organ. The linear relation between the rate of discharge and the tension suggests that the decisive stimulus is the tension exerted on the dendrites. It is of interest that the generator potential is apparently also simply related to the final rate of discharge (Burkhardt, 1959).

However, if the viscous properties of the dendrites differ from those of the rest of the bundle, the tension changes in the dendrites during a quick extension will be correspondingly greater or smaller than the tension changes in the bundle as a whole. It is likely that the steeper relation between firing and receptor tension during the dynamic phase is due to greater viscosity of the dendrites. (A similar explanation was put forward by Matthews (1933) to account for the dynamic phase of discharge of the vertebrate muscle spindle.)

The possibility that stretch causes a temporary depolarization by increasing the membrane capacity of the dendrites (cf. Katz, 1950) seems unlikely in our experiments, in which the rate of stretching was relatively slow compared with the probable electrical time constant of the membrane.

SUMMARY

1. When isolated abdominal stretch receptors of the crayfish (*Astacus fluviatilis*) are stretched in steps, and then released, the changes in tension are not linear, and they show marked hysteresis, as in ordinary muscle.

2. The two kinds of receptors have similar mechanical properties, but the fast-adapting organ has a lower coefficient of elasticity, and so needs a greater degree of extension to produce a given change in tension per unit area. This may account for its high threshold.

3. The steady rate of discharge of the slow-adapting receptor is a linear, and not a logarithmic, function of the tension exerted by the receptor. The peak of discharge of the fast-adapting receptor is also linearly related to the tension.

4. Immediately after stretching both the tension and the discharge show a dynamic phase (or overshoot), which can be ascribed to viscous elements in the receptor organ.

5. The quicker adaptation of the fast-adapting receptor is probably not a consequence of its mechanical properties.

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*Note added in proof.* In a recent paper L. Wendler & D. Burkhardt (1961; Zeitlich abklingende Vorgänge in der Wirkungskette zwischen Reiz und Erregung (Versuche an abdominalen Streckreceptoren dekapoder Krebse). *Z. Naturf.* **16***b*, 464–469) describe measurements of the relation between the discharge and the tension of crayfish stretch receptors and also some effects of electrical polarization. Although their observations in general agree with ours, they conclude that adaptation of the receptor neurone is partly responsible for the decay of activity after the peak of the dynamic phase of discharge.

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# THE RELATIONSHIP BETWEEN SACCADIC AND SMOOTH TRACKING EYE MOVEMENTS

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Dodge (1903) gave the first comprehensive description of the types of eye movement which can occur. Since that time there has been repeated confirmation that when the eyes follow moving objects they make two types of movement: jerky movements, called saccadic movements, and smooth movements in the intervals between these. Westheimer (1954*a*, *b*) has analysed the saccadic movements and shown them to be the response to simultaneous rapid changes in innervation to the eye muscles involved. The nature of the movement is determined largely by the mechanical properties of the structures in the orbit.

The smooth movements have been described by several workers. Dodge, Travis & Fox (1930) investigated the movements obtained when tracking black and white vertical bars moving horizontally across the visual field—opto-kinetic nystagmus—in man, and also the response to a 'slit' of light moving from side to side with simple harmonic motion; Rademaker & Ter Braak (1948) described opto-kinetic nystagmus in rabbits; Westheimer (1954*b*) investigated the response to the horizontal movement of a single spot of light with uniform velocity, harmonic motion, random motion, and with uniform velocity exposed intermittently.

There are three questions which have not been satisfactorily answered by these investigations on tracking movements. First, what decides whether the eyes should perform a saccadic movement or a smooth movement? Secondly, in what way are smooth movements metrically related to the movement of the target? Thirdly, are saccadic movements and smooth tracking movements different modes of action of the same neurological apparatus, or are different pathways involved?

What decides whether the eyes should perform a saccadic movement or a smooth movement? There is abundant evidence that when the target is stationary and does not lie in the line of sight, fixation is achieved by one or more saccadic movements. Smooth movements are only elicited when the image of the target moves smoothly over the retina. The association between the movement of the image over the retina and smooth tracking eye movements has led to the assertion (e.g. Walsh, 1957) that the move-

ment of the image is the stimulus which evokes smooth tracking, although the evidence is insufficient to justify this conclusion. The evidence available is equally compatible with the view that both saccadic and smooth movements are caused by positional errors in fixation, but that smooth movements occur only when there is no discontinuity in the position of the target. Evidence will be presented here that it is indeed the movement of the image over the retina which is detected and measured, and results in a smooth tracking response.

In what way are smooth eye movements metrically related to the movement of the target? Dodge *et al.* (1930) have shown that the maximum speed of movement of the target which can be adequately tracked is about 30–40°/sec. This has been confirmed by Westheimer. However, no detailed observations have been reported which relate the way in which the final tracking speed is reached from an initial state of rest, to the speed of the target. It will be shown here that the speed of the target determines the rate at which the final tracking speed is reached. Such observations are of importance because they throw light on the way in which information about how the target moves is utilized to produce an appropriate response.

Are saccadic movements and smooth tracking movements different modes of action of the same neurological apparatus, or not? The pathways involved in fixation and tracking have not been successfully identified. It is not clear whether the cortex is necessarily involved in these movements. Stimulation and ablation experiments reported in the literature have given conflicting results, and the nature of the movements is rarely reported. The possibility exists that some information can be gleaned from the way drugs influence tracking responses. It will be shown that saccadic and smooth movements may be differentially affected by drugs.

This paper presents a re-examination of the eye-movement responses to horizontally moving targets, with particular emphasis on these three questions.

## METHODS

The details of the method of recording eye movements have been reported elsewhere (Rashbass, 1960). The technique is essentially photo-electric, and has a precision of about 5′ and a time of response of 5 msec. The apparatus was calibrated by the repeated fixation of a series of five points separated by intervals of 1°.

The subject sits in a totally darkened room. The position of the head is fixed by a moulded bite-bar, a moulded forehead rest, and a firm rubber bar behind the occiput. The left eye is covered, and both the viewing and recording are carried out with the right eye. The tracking target is a sharply defined spot, 1 mm diameter, on the face of a 5″ cathode-ray tube, placed 1 or 2 m away from the subject. The phosphor is blue with negligible persistence. All movements of the target are effected silently and without warning. The experiments were performed on four young emmetropic subjects.

RESULTS

*Experiment* 1. The spot at which the subject was looking was moved suddenly to a new position horizontally displaced from the original position. The magnitude (0·1–5·0°) and direction of the movement were varied irregularly. The results confirm previous observations that each change in the target position is followed after a reaction time of 150–250 msec by a saccadic movement which directs the eyes towards the new position of the target. This saccadic movement generally matches the target movement to within 0·2°, but occasionally it does not, in which case it is followed after another reaction time by a further saccadic movement. In some subjects there may be three or four saccadic movements, each separated from the previous one

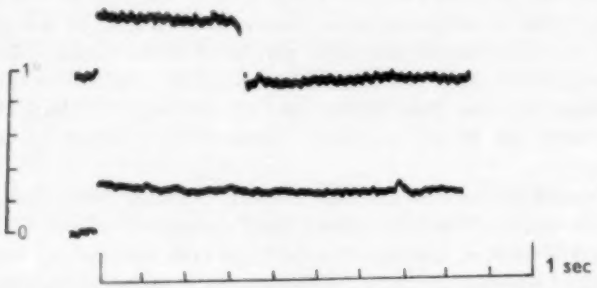


Fig. 1. Successive records of horizontal eye direction after the sudden displacement of the target near threshold. The upper record shows a saccadic response to a target movement of 0·4°; the lower record no response to a target movement of 0·3°. At the moment the target spot moves a displacement is imparted to the record equal in size to the displacement of the target. This displacement can be seen at the left-hand end of the records.

by approximately one reaction time, before the final fixation position is reached. Thereafter small saccadic movements occur around the point of fixation, but the pauses between these are usually much longer than a reaction time. Occasionally these spontaneous saccadic movements occur in trains of two or three with only a short interval (100–150 msec) between the movements.

An observation made in the course of these experiments, which has not been reported previously in the literature, is that there is a threshold target displacement. Target displacements smaller than this produce no responding saccadic movement. This threshold is about 0·25–0·5° in size. Figure 1 shows a normal saccadic response to a target movement of 0·4°, and no response to a target movement of 0·3°. This does not mean that movements smaller than 0·25° cannot occur, for such small movements

frequently occur spontaneously. This suggests that the threshold is associated with the sensory aspects of the response, rather than with the motor characteristics.

*Experiment* 2. The spot which the subject was fixating was made to move horizontally with uniform velocity from an initially stationary position. The direction and magnitude of the velocity of the movement were varied irregularly. The movements of the eye were recorded during the first second or so of the target's movement. In general the eye movements consisted of a smooth component in the direction of the target's movement, with one or more saccadic movements also in that direction. The time of occurrence of the first saccadic movement was not constantly related to the onset of the smooth movement. Figure 2 shows records taken from one

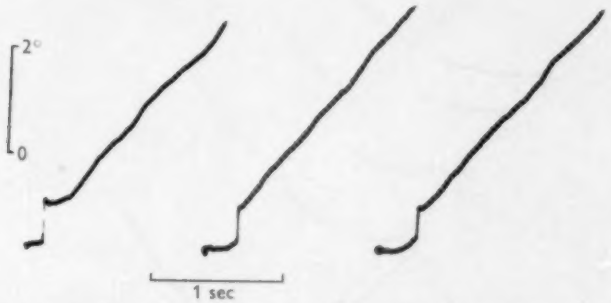


Fig. 2. Records of horizontal eye direction showing the response to the onset of the target's moving with uniform velocity. Note the independence of the saccadic movement from the onset of the smooth movement.

subject during one recording session and illustrates the saccadic movement occurring before, at, and after the beginning of the smooth movement. The appearance of these records suggests that fluctuations in reaction time can occur independently for the two types of movement.

One of the problems under investigation was the metrical relationship between the response and the movement of the target. Preliminary experiments suggested that this relationship was approximately linear; i.e. that at any time after the onset of the target's motion, the distance the eye had moved was proportional to the velocity of the target. The verification of this supposition would require the measurement and re-scaling of many records and so a modified method of recording was adopted. A multi-position switch was used to select from a range of velocities, and at the same time to alter the gain of the recording system in such a way that the product of gain and velocity was held constant. Thus, when the target spot moved slowly and the excursion of the eye was correspondingly

reduced, the recording gain was increased accordingly. If the supposition that the eye movements are linearly related to the target movement is true, all the records obtained by this method will look alike. Figure 3 shows a series of records of the responses to five different velocities over a tenfold range of velocity. These records are from one subject on one occasion. The only selection that has been applied is the rejection of records with an early saccade, as it is felt that the late-occurring saccadic movements allow a clearer view of the smooth component. It can be seen that the records fall into two groups; the three faster-velocity records differ from the two slower-velocity records in that no saccadic movement occurs in the latter.

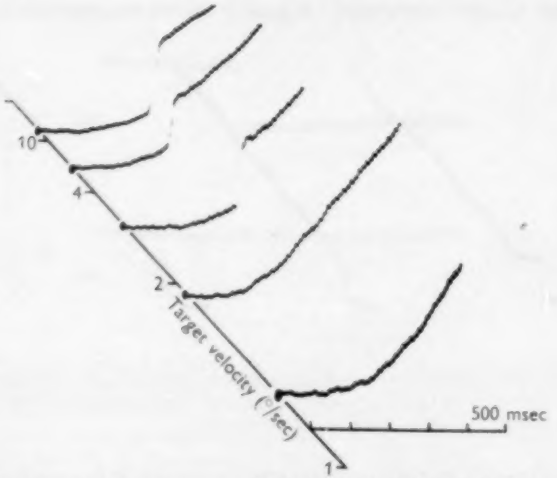


Fig. 3. Records of a series of responses to the onset of a uniform velocity movement of the target. The target was moved at various velocities. Time is displayed horizontally, eye position vertically, and target velocity along a third axis at 45°. The gain of the recording system is made inversely proportional to the target velocity.

The saccadic movements in the three faster records are similar deflexions. Because of the dependence of gain on target velocity, the actual magnitude of the saccadic movements is smaller the slower the target velocity. The saccade in the response to the 3°/sec target velocity is 0·6°. If there were a similarly-appearing saccade in the next slower record it would represent a movement of somewhat less than 0·4°. Its absence is therefore compatible with the threshold for saccadic movements which has been noted earlier.

The smooth movement in all five records appears substantially the same. There is some flattening of the smooth movement in the fastest record (12·5°/sec) and this tendency is more striking in records taken at even

greater velocities. However, within the range from 10°/sec down to the slowest velocities which produce significant eye movements within 250 msec of the target moving, about 0·8°/sec, the smooth movement appears linearly related to the target movement.

The smooth movements begin after a reaction time of about 150 msec, and do not reach the same speed as the target until about 400 msec has elapsed since the target began moving. The smooth movement alone must therefore inevitably leave the point of fixation of the eye trailing behind the target even when the eye and the target are both moving at the same

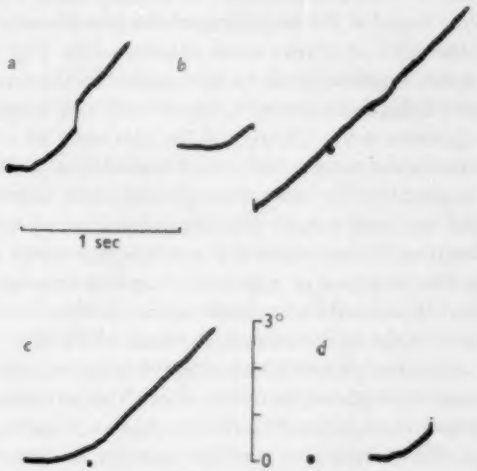


Fig. 4. Tracking responses to a target moving with uniform velocity preceded by a variety of displacements. (*a*) no displacement; (*b*) 3° displacement in a direction opposite to the velocity; (*c*) 1° displacement in a direction opposite to the velocity; (*d*) 1° displacement in the same direction as the velocity. Note the similarity of the smooth component in all cases.

speed. This lag is corrected by saccadic movements in much the same way as an error in fixation of a stationary target.

*Experiment* 3. The next problem under investigation was whether the smooth movements are brought about by the position of the target's image on the retina, or by its movement over the retina. It is possible to perform a crucial experiment by imparting to an initially stationary target a displacement to one side, and at the same time beginning a movement of uniform velocity towards the opposite side. If smooth movements are the result of positional displacement of the target the movements will be towards the target, but if the smooth movements are brought about by the movement of the target they will be away from the target. To this end the

target spot was made to move in the following manner. From an initially stationary position the target was suddenly displaced horizontally to a variable extent and in either direction irregularly. At the same time the target was made to move with a velocity of 3·5°/sec irregularly in either direction. Figure 4 shows records taken in the course of this experiment. The movement of the target supplies the time base of the records and the eye position is represented vertically. Recording was started just before the target began to move, so that a dot appears on each record and indicates the initial positions of the target and eye.

Figure 4*a* shows a record obtained in a case where there was no displacement of the target at the beginning of the movement. It is therefore a repetition of the sort of observation illustrated in Fig. 3, and shows a smooth movement supplemented by a saccade. In the case of Fig. 4*b* the target was given a displacement of 3° to one side and a velocity of 3·5°/sec towards the opposite side. Clearly, if the eye were at all times looking accurately towards the target, the record would be a straight line sloping upwards at approximately 45° through the spot marking the initial positions of the eye and target. The interpretation of the record is that after a reaction time during which the eye does not move, a smooth movement starts in the direction in which the target is moving. When this has been established, a saccadic movement occurs in the direction opposite to the smooth movement to counteract the lead which the eye has over the target. Thereafter the eye continues to move with the target, maintaining accurate fixation throughout the next 1·5 sec. This result indicates that the smooth movement is stimulated by the movement of the target irrespective of its position. The conclusion that the smooth movements are brought about by the movement of the target explains the apparently paradoxical observation that the first movement which the eye makes may take the point of fixation further from the target than if no eye movement at all were to occur.

The results of the same experiment also contain two further observations which are of interest. It has been seen that when a target moves with uniform velocity from its initial stationary position, a saccadic movement is made in the direction in which the target is travelling (Fig. 4*a*). It has also been seen that if the target is given a large (3°) displacement to one side and a simultaneous velocity towards the other side, a saccadic movement is made in the direction opposite to that in which the target is travelling (Fig. 4*b*). This suggests that an intermediate size of displacement might exist, which, when accompanied by a simultaneous velocity towards the other side, produces no saccadic movement in the response. This is the case, and it is illustrated by the recording shown in Fig. 4*c*, where the displacement is about 1°. This balance point is not difficult to

achieve, because the threshold for saccadic movements (about $\pm 0{\cdot}5°$) allows plenty of leeway.

There remains one combination to be examined. This is where the displacement and velocity are both in the same direction. The eye movement corresponding to this target movement is shown in Fig. 4*d*. A normal smooth movement is followed by a large saccadic movement, only part of which is seen on the record.

The four records of Fig. 4 show the responses to a target's moving with uniform velocity accompanied by a displacement which varies in magnitude and relative direction. The responses consist of essentially the same smooth movement, on which is superimposed a saccadic movement which varies in magnitude and direction correspondingly. The inference is that the smooth movement is stimulated by the velocity of the target, and the saccadic movement is stimulated independently by the position of the target.

*Experiment* 4. The results of the previous experiments suggest that saccadic movements and smooth movements satisfy independent requirements and are generated independently of one another. Thus it has been seen that they have independent fluctuations in reaction time (Fig. 2), have different thresholds (Fig. 3), and may be in opposite directions (Fig. 4). Further evidence for their independence would exist if it were possible to dissociate the types of movement pharmacologically. Many drugs, e.g. alcohol and methylpentynol ('Oblivon'; British Schering), are said to cause oculomotor disturbances as side effects, the barbiturate drugs being notorious in this respect. The term 'barbiturate nystagmus' has been used for these effects, which have been described in some detail by Bergman, Nathanson & Bender (1952). Moderate doses of barbiturate drugs are said to disorganize fixation by the occurrence of random movements. Sideways deviation of the eyes whilst under their influence is characterized by a nystagmus, the slow phase of which is towards the straight forward direction. The barbiturates are therefore the drugs of choice with which to start an investigation into the possible pharmacological dissociation of the two types of eye movement. Figure 5 shows the records of the eye movements while tracking a target moving with uniform velocity before and after the intravenous administration of sodium thiopentone (Pentothal; Abbott Laboratories) 100 mgm. The drug was made up in a concentration of 1·25 g/100 ml. and administered continuously at the rate of 1 ml./min. Records of tracking a target moving at 3·5°/sec were taken at frequent intervals during the administration of the drug. The first noticeable effect was the increase in the number of saccadic movements occurring during the first second of tracking. As the amount of drug given increased, the saccadic movements increased at the expense of the smooth movement, until,

after 8 min, no smooth tracking movement could be detected. Figure 5 is a record taken at this stage.

The most likely interpretation of this record is that the drug interferes with the smooth tracking response so as to make it inoperative. However,

Fig. 5. Tracking eye movement responses to a target moving with constant velocity (*a*) before and (*b*) after the intravenous administration of sodium thiopentone (Pentothal) 100 mg.

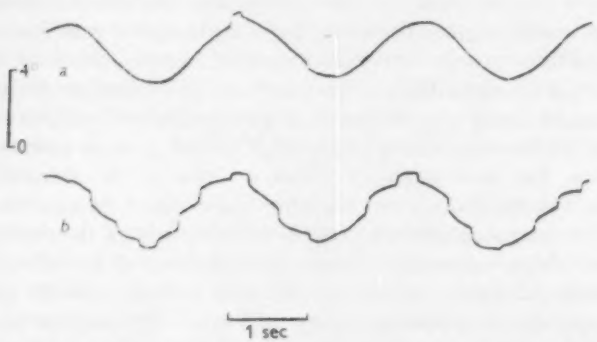


Fig. 6. Tracking eye movement responses to a target moving with simple harmonic motion (*a*) before and (*b*) 100 min after the oral administration of amylobarbitone sodium (Amytal) 300 mgm.

there is another possible interpretation which needs to be eliminated before this is established. If the barbiturate drugs acted by producing a nystagmus which happened to have the slow phase in the direction opposite

to the tracking movement, and the velocity of the slow phase of the nystagmus were just equal to the velocity of the smooth tracking movement, and the nystagmus and the tracking movement combined additively, then a record something like that illustrated would result. That this is not, so was clear from the fact that the records looked the same whichever the direction of the tracking. A more convincing demonstration of this is available in Fig. 6. This shows the effect of a barbiturate drug, in this case amylobarbitone sodium (Amytal; Lilly) 270 mg taken by mouth, on the ability to track a sinusoidally moving target. The target was moving from side to side with a frequency of 0·5 c/s and an amplitude of 4°. Before the drug was taken this movement was tracked smoothly with very few saccadic movements. One hundred minutes after the drug was taken tracking was effected mainly by a succession of saccades.

## DISCUSSION

The experiments reported in this paper show that the eye movements which are made in tracking consist of the superimposed movements of two independent systems. This is not an example of redundancy in the central nervous system for the mechanisms subserve different demands. The smooth movements are responses to movement of the image across the retina, and in the absence of saccadic movements could do nothing to bring the image of the target on to the fovea. On the other hand, the smooth movements do result in a stationary image being cast on the retina by a moving target, in much the same way as when a camera is slewed round to photograph a moving object.

Not only are smooth eye movements brought about by the movement of the image over the retina, but the velocity of the smooth movement is linearly related to the velocity of movement of the target. This means that the velocity of the image on the retina is not only detected but also measured with considerable precision over a fairly wide range of velocities. The measurement of velocity could be performed on the retina or more centrally. It has been argued that if the velocity of the image is the stimulus which evokes smooth tracking responses, the ultimate speed of movement of the eye must always be slightly less than the speed of movement of the target. This deficiency has never been demonstrated, except in the case of very fast moving targets, and it has been suggested that this is due to the inadequacy of recording techniques. However, it can be shown that there need be no residual movement of the image over the retina to maintain the movement of the eyes. If a movement of the image on the retina evokes an acceleration of the eyes in the direction of that movement, then the eyes will only travel with uniform velocity (i.e. without acceleration) if there is no image movement. This appears to be the way in which a precise match

of eye velocity to target velocity is achieved. It is known that very slow drifts in eye position occur even during fixation of a stationary target. The error in the velocity of tracking a uniformly moving target does not exceed the velocity of these drifts.

Westheimer (1954*a*) has pointed out that the saccadic movement is essentially ballistic. This means that the decision concerning the magnitude of the movement is taken before the movement begins, and that the movement then follows an inevitable course. The adequacy of the movement is tested in retrospect, and any deficiencies are rectified by similar ballistic movements. The alternative to a ballistic movement is a guided one. Such a movement is under continuous control, and should the movement at any time be found to be inadequate, either because of its own inaccuracy or because of changes in the task requirement, it can be modified accordingly. The question therefore arises whether smooth tracking movements are ballistic or guided. Westheimer (1954*b*) has suggested that smooth movements are also ballistic. His reason for saying this is that he describes smooth movements as consisting of stretches of movement of uniform velocity joined by very short regions during which the velocity changes. The changes are said to last not more than 20 msec and to recur not more frequently than every 100 msec. This view is in agreement with the observation by Stroud (1950) that when tracking harmonic motions many cycles need to elapse before smooth accelerations are found in the responses. On the other hand Dodge *et al.* (1930) have described and illustrated smooth accelerations occurring within half a cycle (0·4 sec) of the onset of the oscillations, and Westheimer (1954*a*) has shown a change in velocity which occurs gradually over a period of 90 msecs. The records reproduced in the present paper suggest that it is difficult to be sure on this point. Figure 5 shows a sudden change of velocity, but the accelerations in Fig. 3 appear to occur gradually and slowly. The remaining illustrations show all graduations from very pointed to very gradual.

It is clear that the ability to distinguish sudden from gradual accelerations depends on the precision with which the eye position can be ascertained, for small deviations may make a smooth acceleration appear sudden or a sudden change of velocity appear gradual. The variance in eye position during fixation of a stationary target is about 1/10° (Barlow, 1952) and this may be taken as the noise level below which it is profitless to look for significant tracking movements. The distinction between sudden and gradual accelerations is equivalent to the distinction between a triangular and a sinusoidal motion, and depends on the detection of harmonic components, the largest of which is 1/9 the amplitude of the fundamental oscillation. From these figures it can be shown that a change of velocity of $V°$/sec cannot be localized in an interval much shorter than $2/V$ sec. It

would seem therefore that the question whether smooth eye movements are ballistic or guided cannot best be decided by the presence or absence of corners in the records.

The distinction between a guided system and one which makes successive approximations with a series of ballistic movements is to a certain extent arbitrary. It may be assumed that a system takes in information at discrete intervals which, in the limit, approaches continuous sampling. The response of the system to one sample of input information will extend over a certain time, which we may call the response time. If the sampling interval is large compared with the response time, then the system will be described as ballistic. Saccadic eye movements fall in this category, as do, for example, nerve action potentials. In the latter case the sampling interval is determined by the refractory period, which exceeds the response time, i.e. the duration of a spike. If, on the other hand, the response time is large compared with the sampling interval the system would be considered to be guided. Muscle contraction provides a good example of such a system. Clearly it is possible to have any degree of guidedness between the extremes, and it is doubtful whether the distinction is always worth making.

In view of the narcotic action of the barbiturate drugs it would be tempting to attribute the action which they have on tracking to a reduction in the attention which the subject gives to the task. This is not so, for a subsidiary experiment showed that nitrous oxide mixed with air in sufficient concentrations nearly to produce unconsciousness has very little effect on eye movements. So long as the subject can keep his eyes open he is able to track without impairment. The effect of barbiturates on the smooth component of tracking movements is complete with doses which do little more than induce slight drowsiness. The effect of barbiturates on tracking is, therefore, not secondary to their narcotic action, but is more direct. Together with information concerning the effect of barbiturates on other oculomotor functions (Rashbass & Russell, 1961; Westheimer & Rashbass, 1961) this helps to delimit the site of action of the drug and to distinguish the neurological pathways involved.

## SUMMARY

1. Tracking eye movements were recorded in normal subjects presented with a variety of visual tracking tasks.

2. Sudden lateral displacements of the target evoke saccadic responses. Displacements smaller than about 0·25° cause no response.

3. The initial response to a target moving with uniform velocity has both saccadic and smooth components.

4. The reaction times of these two components vary independently.

5. The smooth component is linearly related to target velocity up to 10°/sec; the saccadic component has a threshold.

6. Tasks involving various combinations of displacement and velocity evoke saccadic and smooth responses; the smooth movement is determined by the task velocity, the saccadic movement by the task displacement.

7. The barbiturate drugs differentially interfere with the smooth-movement component.

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# DISJUNCTIVE EYE MOVEMENTS

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Eye movements may be described in terms of two components: conjugate and disjunctive. A pure conjugate eye movement is one in which the two eyes move in a parallel fashion, while in a disjunctive movement changes occur in the orientation of one eye with respect to the other. A typical situation calling for a disjunctive movement is the change in binocular observation from a far to a near target, when the fixation lines of the two eyes have to converge.

Recordings of eye movement responses to the presentation of targets requiring convergence show the movements to be slow compared with their reaction time (Westheimer & Mitchell, 1956). In this respect they differ from the most commonly occurring conjugate eye movements, the saccadic movements. It is clear now that saccadic eye movements are not guided, in that no adjustments in response to visual clues are made during the movement (Westheimer, 1954). The time characteristics of disjunctive movements, on the other hand, would theoretically allow correction of movements during their course on the basis of visual information. That the elements for such a control system exist is evidenced by the following two facts: (1) the sensory system on which such a control system would depend is capable of making very fine discriminations—retinal disparities of less than a minute of arc can be detected—and (2) the muscular system is quite fast and capable of making fine gradations in response. The experiments reported in this paper fall into two groups. Those describing the eye movement responses to simple types of convergence stimuli are given in Part I, and those in which responses are studied to special stimulus situations designed to elucidate the properties of the control mechanism for disjunctive eye movements are contained in Part II.

## PART I. DESCRIPTIVE EXPERIMENTS

### METHODS

*Stimulating.* The subject sits in a darkened room. Each eye is presented with a target consisting of a 3 mm white spot on the face of a large cathode-ray tube 2 m from the eye.

* On leave from the Institute of Psychiatry, University of London.

One of these tubes is placed directly in front of the subject and is seen by only the left eye. The other is placed to one side and is seen by only the right eye after reflexion in a front-surface mirror. This mirror also obscures the right eye's view of the left eye's target. The

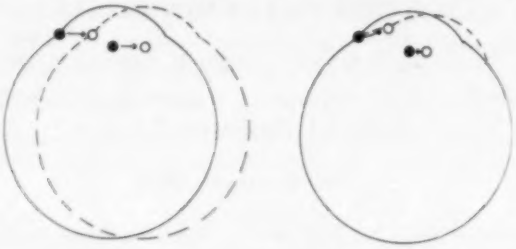


Fig. 1. Schematic diagram to illustrate relative changes in the positions of the corneal light reflex and a point on the limbus as the eye undergoes (*a*) a side-to-side translational movement and (*b*) a rotational movement.

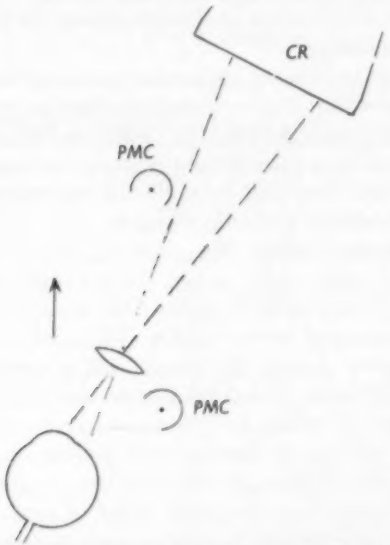


Fig. 2. Schematic plan of the experimental arrangement to record eye movements. A vertical line scans the cathode-ray tube (*CR*) horizontally and is imaged by the lens on the anterior surface of the eye. Two photomultiplier cells (*PMC*) are situated in front of the eye to register changes in the reflected light.

cathode-ray tube spots may be deflected horizontally with applied voltage signals. When equal signals are applied to both tubes, this results in a symmetrical disjunctive movement of the targets.

*Recording.* The movements of the eyes are recorded by a method which is designed to eliminate any artifacts which may arise from lateral displacements of the eyeballs. The

method is based on the principle that, seen from one direction, the separation of two points on a rigid body remains constant during a translation but changes during a rotation around an axis normal to the plane containing the two points and the direction of observation. The two points chosen which to a first approximation have this property for horizontal eye movements are the temporal limbus and the image of a light source by specular reflexion in the anterior surface of the cornea. Seen from the front, a point in the limbus will move through the same lateral distance as the corneal reflexion of a distant light source when the eye makes side-to-side translational movement. When the eye makes a rotational movement around a vertical axis, however, these two landmarks will move through unequal lateral distances. The separation of a point on the limbus from the corneal reflexion will, therefore, be a function of rotational eye position only, unaffected by translational shifts of the eye (Fig. 1).

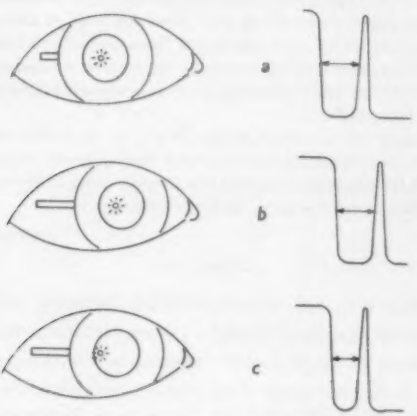


Fig. 3. Summed signals from the two photomultiplier cells in Fig. 2 (schematic). The first down turn indicates the position during the scan in which the image of the cathode-ray tube line travels across the limbus, the rise and fall show the position in which a specular reflexion from the cornea is formed at the second photomultiplier cell. When the eye makes a side-to-side translational movement (*b*) the two changes are displaced in a parallel fashion, but during an eye rotation (*c*) their separation changes. The separation, isolated electronically, is used as the signal corresponding to eye position.

We use a vertical line (produced by 60 kc/s vertical scanning of the spot) on a short-persistence cathode-ray tube and image it by means of a lens on the anterior surface of the eye. The line is made to scan the tube face horizontally in a saw-tooth manner at the rate of 300 c/s. The image is about 1 mm high and moves nasally from the sclera across the cornea at the level of the widest horizontal diameter of the cornea. The light reflected from the anterior surface of the eye is measured by two suitably placed photomultiplier cells (Fig. 2). During a single scan the output of the one cell undergoes a change when the image crosses from the sclera to the cornea, and that of the other detects the instant a specular reflexion of the image from the cornea is formed at this particular cell. In the summed output of the two cells the time interval of the two changes is a measure of the rotational eye position (Fig. 3). The time interval is isolated electronically and a signal proportional to eye position is available. The apparatus is duplicated before the other eye, and records can be obtained of the horizontal movement of each eye separately or, by electronic differencing, of convergence.

The gain of the recording system was so adjusted that the voltage output of each eye's channel was equal to the voltage required to drive each stimulus spot on its cathode-ray tube through a distance corresponding to the movement of the eye. Linearity was demonstrated by the maintenance of this correspondence over the range of eye movements used by us, up to 8°. Translational movements are about five times less effective than if the limbus position alone had been recorded (Rashbass, 1960) and about ten times less than if the corneal reflexion method had been used. The noise level remains under 5 minutes of arc. The frequency response of the recording system is estimated to be good from zero to at least 30 c/s.

*Procedure.* The subject is placed before the experimental apparatus with his head held firmly in place by means of a bite-bar and a head clamp. The mirror in front of the right eye is adjusted so that when each spot is in the centre of its viewing tube face, the subject sees a single spot in binocular view somewhere near the middle of the angular range within which he can maintain single vision (fusional range). Near the edge of this range quite different responses might be found; we have not analysed these nor have we attempted to analyse phenomena close to the noise level of the recording apparatus. Preliminary tests showed that such factors as size, brightness and sharpness of the viewing spots do not materially influence the characteristics of the responses.

*Nomenclature.* We shall use the term 'target vergence' to denote the angle between the lines joining the centre of rotation of each eye with the apparent target for that eye. 'Eye vergence' will be used for the angle between the fixation lines of the two eyes. 'Disparity' is the difference between target vergence and eye vergence.

## RESULTS

Records of disjunctive eye movements in response to horizontal target vergence stimuli were obtained in eight young adult subjects, none of whom showed any evidence of oculomotor imbalance, as determined by tests for heterophoria and fusional range. The results described below are typical of the records obtained on all subjects. Variation between subjects, except where mentioned, appeared negligible. Except where noted, movements in the direction of convergence and divergence had similar characteristics. The targets remained at the same physical distance throughout, so that any additional effects that a real near target might introduce, such as 'cross-talk' from accommodation, were avoided.

It is clear now that the response mechanisms mediating conjugate and disjunctive eye movements operate separately (Westheimer & Mitchell, 1956; Yarbus, 1957; Rashbass & Westheimer, 1961). This means that the method of display adopted here, in which only the disjunctive component of the binocular eye movement is shown, is a sufficient measure of the phenomena studied. In any case, when the target vergence is introduced symmetrically before the two eyes, as was done throughout, the conjugate components of the eye movements are small.

### *Step stimuli*

Figure 4 shows a representative eye vergence response when a 2° target vergence change is suddenly introduced. The reaction time is typically

about 160 msec. A constant velocity of eye vergence is soon developed and maintained for nearly 200 msec, after which the velocity falls off. The final level of eye vergence is reached in about 800 msec, so that in total about 1 sec elapses between the onset of the stimulus and the stabilization of the response at the new level of eye vergence. These time characteristics of step vergence responses are similar to those of accommodation (Campbell & Westheimer, 1960).

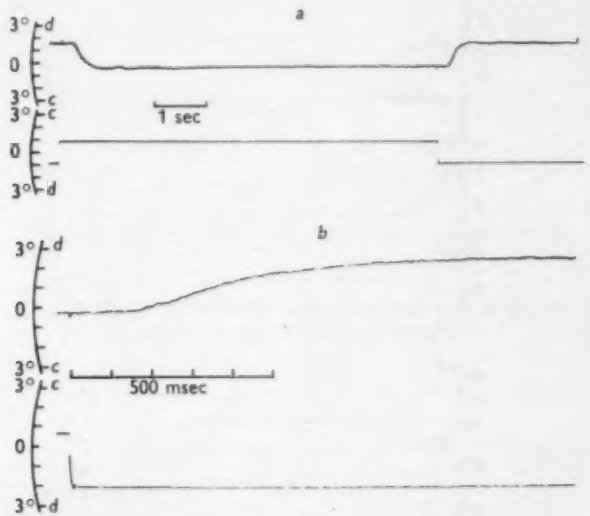


Fig. 4. (*a*) Eye-vergence response (upper trace) to a step change in target vergence of 2°. Allowance should be made for the arc of the pen. The direction of convergence is marked *c*, and of divergence, *d*. (*b*) Same, but at a higher recording speed.

Figure 5 shows responses to step stimuli of various amplitudes with the gain of the recording system changed to compensate. Characteristic changes occur as the amplitude of the step target vergence stimuli is decreased. The larger movements are slowed relatively earlier in their course and reach their final level asymptotically. The 20′ movement, on the other hand, has an overshoot in one direction.

The amplitudes of the eye vergence movements match the target vergence changes very well. It appears that if there is any maintained disparity it certainly does not exceed a few minutes of arc, a fact that has also been demonstrated in recent experiments with the contact-lens method of recording eye movements (Riggs & Niehl, 1960).

Figure 6 shows a 3° movement recorded from subject E.F.M. This particular subject shows oscillations for movements in one direction not

only around the final position but also during the movement. We shall see similar oscillations in another subject when ramp stimuli are illustrated below.

Some details of the time course of the responses shown here differ from those given by Westheimer & Mitchell (1956). Genuine individual differ-

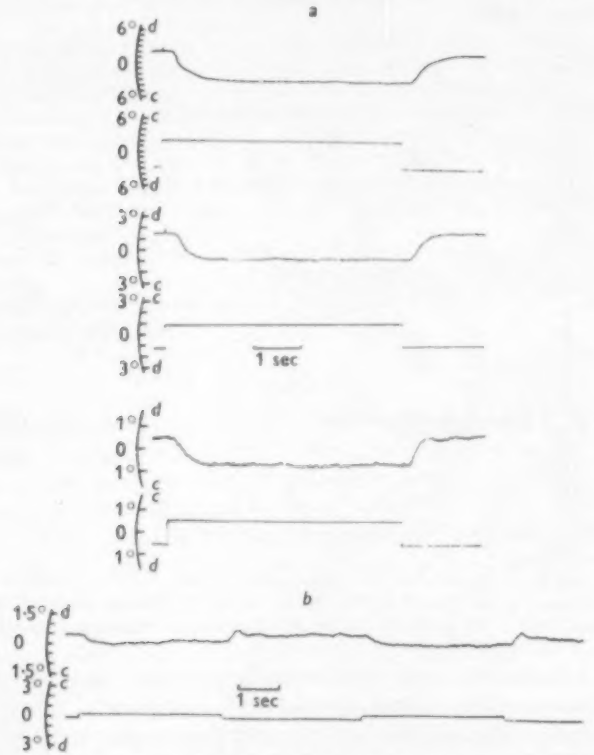


Fig. 5. Eye-vergence responses to step changes in target vergence of (*a*) 4°, 2°, 1°, and (*b*) 20′. Note changes in vertical scale.

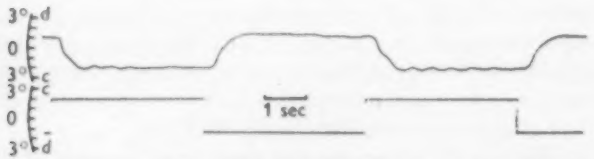


Fig. 6. Eye-vergence response to a 3° step change in target vergence from subject E.F.M.

ences might be involved, but it is more probable that the improved recording technique available for this study accounts for the discrepancies.

Are the responses shown in Figs. 4 and 5 initiated as a single event and carried through without modification as are, for example, saccadic eye movements, or are the particular response characteristics the result of changes introduced during the course of the movements? The following experiment suggests an answer to this question.

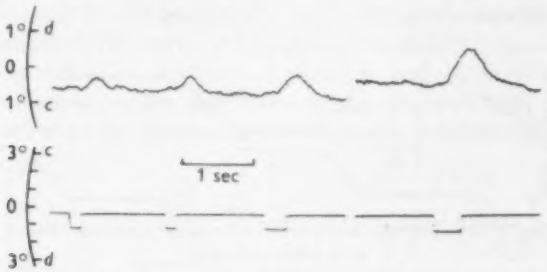


Fig. 7. Eye-vergence movements in response to 1° pulses of target vergence of various duration.

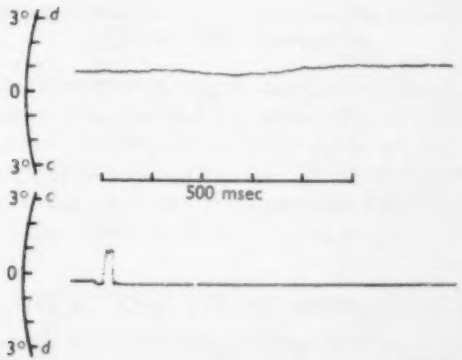


Fig. 8. Response to a 2° target-vergence pulse of 20 msec duration.

*Rectangular pulse stimuli*

In Fig. 7 are shown the responses to a series of sudden target vergence changes followed in each case by a return of the stimulus to the original level after a time interval increasing throughout the series. It is seen that there is an ascending and a descending limb in each response, the two separated by an interval approximately equal to the pulse-stimulus length; the response lags a reaction time behind the corresponding components of

the stimulus. It is concluded that disjunctive eye movements can be modified during the course of their progress and that information concerning disparity can be assimilated during the reaction time as well as during the movement.

The initial responses to constant-amplitude target vergence pulses of various durations can all be superimposed on each other as well as on the step response to the same stimulus amplitude.

The shortest pulse we have used was 20 msec long. Figure 8 shows that there is a response.

Fig. 9. Eye-vergence movements when target vergence changes at constant velocity.

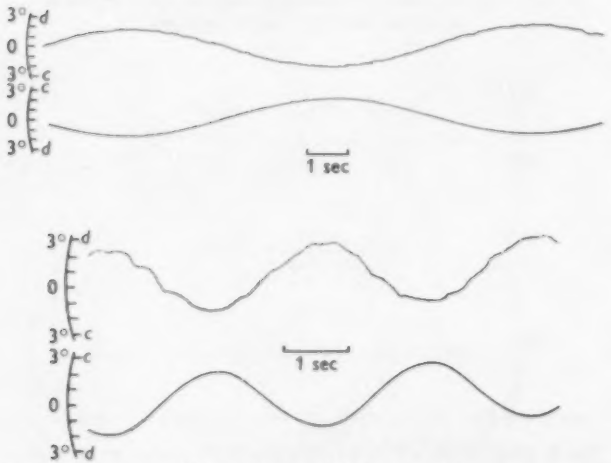


Fig. 10. Eye-vergence tracking of sinusoidal changes in target vergence at two frequencies. The records shown are from the middle of long trains of movements and do not exhibit transient changes associated with the beginnings and ends of such responses.

### *Ramp functions*

When a target vergence is introduced with a constant rate of change (Fig. 9), it is matched by a rate of change of eye vergence, but this is not achieved without some velocity overshoot and oscillations. Typically these

transient effects are unequal in the two directions. A reaction time of somewhat less than 200 msec is a feature of all these records. We have recorded tracking of target-vergence changes as slow as 0·35°/sec and as fast as 5°/sec.

*Sinusoidal target vergence stimuli*

The targets were made to undergo vergence changes that were a sinusoidal function of time at various amplitudes and frequencies. That the vergence tracking is exceedingly accurate for stimuli with periods of a few seconds per cycle can be seen in Fig. 10, which shows records during the middle of long trains of waves. When the amplitude and frequency are such that tracking velocities of several degrees per second are demanded, the velocity oscillations—more prominent in one direction than the other—already observed in the previous section are again demonstrated. When the

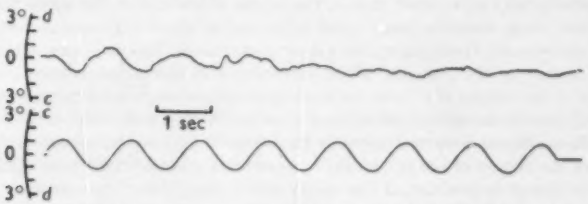


Fig. 11. Gradual deterioration of eye vergence tracking of fast (1 c/s) sinusoidal target vergence movements.

frequency of the sinusoidal stimuli is increased to the order of 1 c/s, the subject responds to only the first few cycles (Fig. 11); the response then fluctuates irregularly in the middle of the stimulus range. Under these conditions the subject is aware of diplopia. This phenomenon is dependent to some extent on the amplitude of the stimulus oscillations, but we have not investigated this aspect in detail.

## PART II. ANALYTICAL EXPERIMENTS

In the preceding part eye-vergence responses to a variety of target patterns have been described. In this part investigations are reported to determine which characteristics of the disparity determine the nature of the response, and the way they do so.

An analysis of this sort is best performed by observing the responses to disparities that are simple time functions entirely under the control of the experimenter. In the experiments described in the preceding part this is not the case. There the binocular disparity results from the combination of the movements imposed on the targets with the movements which the eyes perform. The eye movements themselves are not directly under the

control of the experimenter and thus any attempt to impose a simple disparity is vitiated as soon as eye-vergence movements are made.

It is possible to surmount this difficulty by making the target spots move in a way that is predicated by the eye-vergence movements. With the apparatus described the target spots can be driven by the electrical measure of eye vergence so that any vergence movements made by the eyes are accompanied by similar movements of the targets. When this is achieved, eye vergence no longer has any effect on disparity. It is possible to introduce any desired disparity which then will remain uninfluenced by the response it evokes. This process may be termed disparity clamping and is familiar to engineers as the process of opening a feed-back loop.

## METHODS

It has already been mentioned that in the course of calibration the signal from the eye-movement recording device is made equal to the signal which displaces the target so as to evoke that movement. The input to the target presentation devices is now earthed to bring both spots to their central position. When convergence on this target position is established, the DC level of the output of the eye-vergence measuring system is adjusted to earth potential. This adjustment is carried out without altering the sensitivity which has already been adjusted. It is now possible to disconnect the input to the target presentation devices and replace it by the output of the eye-vergence measuring system (which is at earth potential) without any change in position of the target spots. Should any eye-vergence movements now occur, the target spots will move so as to keep the disparity zero.

The substitution of the real earthing connexion of the target presentation devices by the output of the eye-vergence measuring device changes the stability of the system from a condition of stable to one of neutral equilibrium. After the substitution there is nothing to prevent the eye vergence from drifting, because the eye will be accompanied by the targets. With care and patience, however, it is possible to achieve a substantially drift-free base line.

It should be noticed that this procedure does not produce a stabilized retinal image. Conjugate eye movements do not appear in the output of the eye-vergence measuring system and are, therefore, not imposed on the target movements. The targets are always symmetrically disposed about the mid line.

## RESULTS

Figure 12 is a typical record of the response to a convergent disparity that is suddenly imposed and subsequently maintained at a constant value. After a reaction time of 170 msec the eyes begin to converge. The movement almost instantly attains its velocity which is then sustained throughout. The movement ends either when one of the target spots goes out of sight or when the degree of convergence attained is extreme. Divergent disparities cause divergence movements which are of similar character to the convergence movements caused by convergent disparities.

Unlike the records in the previous part, those in this part show the disparity stimulus and the response at different scales. Thus it is seen that when the disparity is clamped, a small disparity evokes a movement which becomes very much greater than the disparity which causes it.

The velocity of the movement varies with the disparity. Figure 13 shows the relationship between these two quantities. Within the limits of the experimental method there is no dead zone, that is to say, dis-

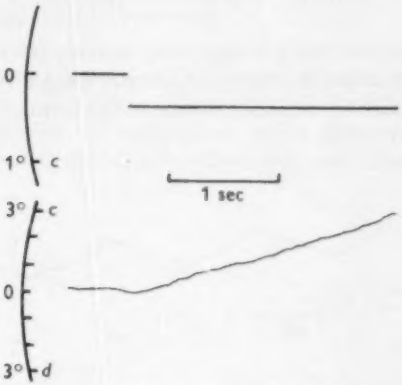


Fig. 12. Eye-vergence movement when a constant disparity is suddenly imposed and subsequently maintained. The velocity of the eye vergence is a function of the degree of disparity.

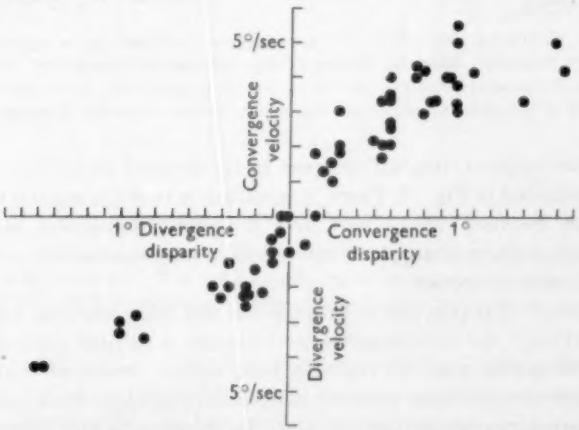


Fig. 13. Relation between the magnitude of constantly maintained disparity and velocity of eye vergence induced by it. Each point was obtained from a record such as illustrated in Fig. 12.

parities, however small, evoke a response. This underlines the difficulty of obtaining a drift-free base line. Within the range of $\pm 0{\cdot}2°$ of disparity, the velocity of the eye vergence is linearly related to the binocular disparity that evokes it, but for higher disparities there seems to be a falling off

from linearity. In the subject on whom the results of Fig. 13 were obtained the constant of proportionality is 10°/sec per degree of disparity, this figure being obtained from the slope of the results near the origin. In another subject the constant of proportionality was 7°/sec per degree of disparity.

When the clamped disparity is alternated between the convergent and the divergent kind, the response consists of alternating convergence and divergence eye movements of uniform velocity in the form of a triangular wave

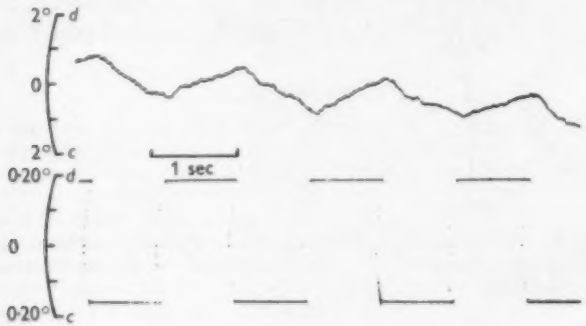


Fig. 14. Triangular wave-form of eye-vergence response to a square-wave disparity stimulus. Note the relatively large movements induced by the small amount of disparity. Drift in the upper record is practically unavoidable consequence of experimental situation required to produce disparity clamping.

delayed one reaction time with respect to the steps of disparity evoking it. This is illustrated in Fig. 14. There is no evidence that the regular repetition of changes between convergent and divergent disparities allows any anticipation of these changes. Even the later responses occur only after a reaction time has elapsed.

At this stage it is possible to propose the following working hypothesis: the velocity of the eye-vergence movements is always proportional to what the disparity was one reaction time earlier, provided that the disparity never exceeds some prescribed bounds. The hypothesis predicts the eye-vergence movements that will occur in response to any pattern of disparity, a prediction that can be tested by observation.

Sinusoidally varying disparities provide a convenient test. The hypothesis may be expressed in the form

$$\frac{dr}{dt} = k\epsilon_{(t-\tau)}, \tag{1}$$

where $r$ is the eye vergence, $\epsilon$ the disparity, $\tau$ the reaction time, and $k$ the

constant of proportionality determined above. For a sinusoidal disparity change of amplitude $a$ and frequency $\omega$ radians/sec:

$$\epsilon = a \cos \omega t;$$

and from equation (1) it follows that

$$r = \frac{ka}{\omega} \sin \omega (t-\tau) = \frac{ka}{\omega} \cos [\omega(t-\tau) - \tfrac{1}{2}\pi], \tag{2}$$

omitting the constant of integration which determines the mean eye vergence. The hypothesis thus predicts not only that the eye-vergence

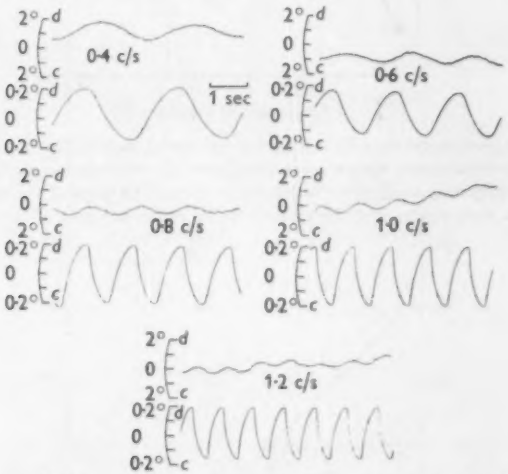


Fig. 15. Sinusoidal eye-vergence movements in response to sinusoidal disparities at various frequencies. This is an experiment in the manner of the 'open-loop' approach to servo mechanisms. Allowance should be made for the arc of the pen.

response to sinusoidally varying disparity will be a sine wave of equal frequency but also says something about the amplitude, and phase relationships between the disparity and the resulting eye vergence.

In Fig. 15 are illustrated sinusoidal disparity changes and the eye-vergence movements they evoke. It is seen that the latter are substantially sinusoidal. The records are from the middle of long wave trains. In Fig. 16 are plotted the amplitudes of the response oscillations against the amplitudes of the disparity oscillations for a single frequency. For small disparities a linear relationship exists between the two, but as the amplitude of the disparity oscillation increases there is a falling off from linearity.

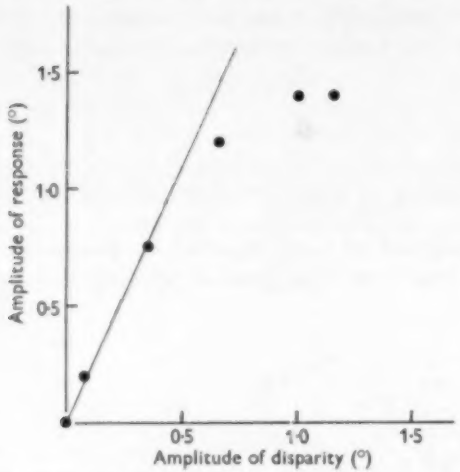


Fig. 16. Experimental data from long wave-trains of records like those in Fig. 15 showing the relationship between the magnitude of eye-vergence oscillations and that of the disparity oscillations which cause them. The slope of the straight line fitted to the data points near the origin may be called the gain of the system.

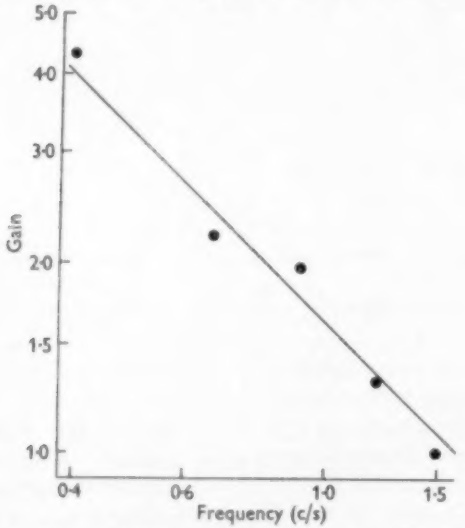


Fig. 17. Graph relating gain (ratio of eye-vergence amplitude to disparity amplitude for sinusoidal disparity changes) to frequency of the disparity changes; log. co-ordinates. Straight line has position and slope theoretically expected in a system in which disparity produces a constant velocity of eye vergence with the relationship in Fig. 13. Points are experimentally determined. Subject C.R.

The ratio of response amplitude to disparity amplitude at any frequency may be called the gain of the system at that frequency. This quantity in its linear region for small disparities has been plotted in Fig. 17 as a function of frequency on logarithmic co-ordinates for the results from subject C.R., obtained from records like those shown in Fig. 15. Superimposed is drawn the predicted line of gain, obtained by substituting in equation (2) the value of $k$ obtained from the experiment illustrated in Fig. 13, that is 10°/sec per degree of disparity. Similar results were obtained for a second subject and are shown in Fig. 18. There is good agreement between the experimentally obtained results for gain as a function of frequency and the theoretically predicted curve.

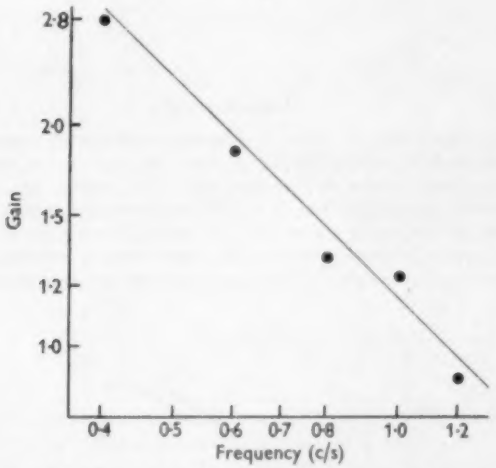


Fig. 18. Results for another subject, B.W.W., similar to those depicted in Fig. 17 for subject C.R. Log. co-ordinates.

Equation (2) also makes a prediction concerning the phase relationship between the disparity oscillations and the response oscillations. Were it not for the delay of a reaction time, the maximum velocity of the response would coincide with the maximum disparity. It is expected, therefore, that the response oscillations will follow the disparity oscillations after a delay of a quarter of cycle plus a reaction time. The straight line in Fig. 19 shows the predicted relationship between phase lag and frequency. The points represent the phase lags measured experimentally, each point being the average of about 100 measurements. It can be seen that there is no agreement between prediction and experiment; the observed phase lags

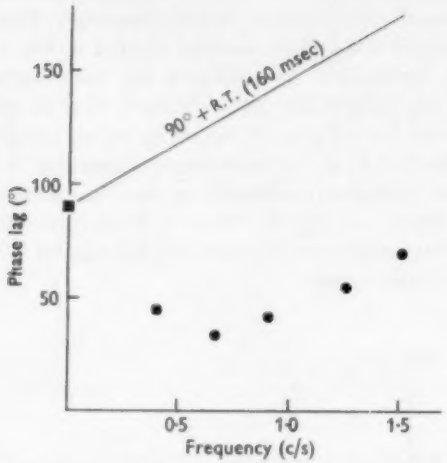


Fig. 19. Relationship between phase of sinusoidal eye-vergence movements and the sinusoidal disparity changes inducing them. Straight line is the expected behaviour of a simple system with characteristics like those in Fig. 12. Points marked ● are from records like those in Fig. 15. Point marked ■ is an expression of the fact that an integrating system (Fig. 12) has 90° phase lag at zero frequency. Measured latencies substantially shorter than expected are an indication of anticipatory behaviour of eye vergence under sinusoidal disparity stimulation. Subject C.R.

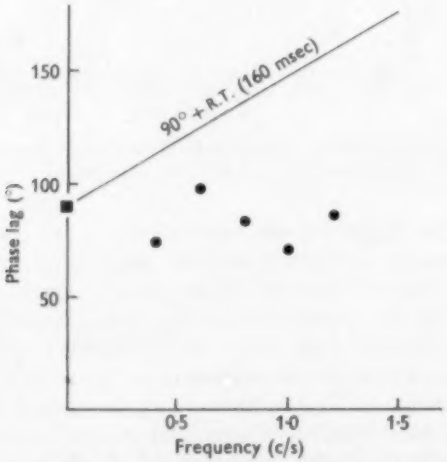


Fig. 20. Results for phase delay between sinusoidal disparity changes and eye-vergence responses for subject B.W.W. See legend for Fig. 19.

are very much smaller than those expected. A similar discrepancy is shown in the results for subject B.W.W. although not quite so markedly (Fig. 20).

There are several possible ways of considering this failure of equation (1) to account for the observed phase lags, yet no simple modification of it would yield a better concordance between obtained and predicted phase lag and at the same time account for the findings illustrated in Figs. 12, 14, 15 and 16.

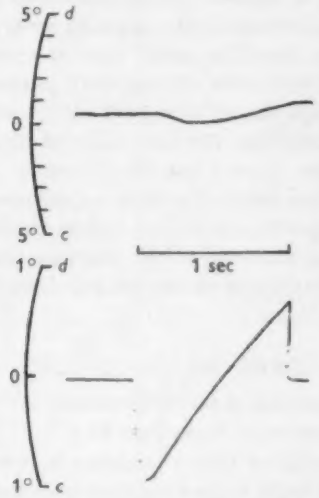


Fig. 21. An experiment to illustrate anticipatory behaviour of the disparity–eye vergence system. The disparity presented would not produce a turn-about in the response until one reaction time after it has crossed the zero level, unless anticipation had occurred.

The fact that the phase delay in the sinusoidal disparity experiment is shorter than would be expected from the hypothesis which accounts for the other findings in Part II suggests that the course of disparity is being anticipated. It might seem reasonable to account for this by the rhythmical repetitiveness of a sinusoidally varying disparity. This explanation, however, is unacceptable because regular alternation of constant convergent and divergent disparities shows no evidence of such anticipation (see Fig. 14). The anticipation of the course of the disparity which is displayed for sinusoidally varying disparities must, therefore, be accountable by some property other than its rhythmicity. The only essential difference between a sinusoidal variation in disparity and a regular alternation between convergent and divergent disparities is that the former is carried out

with finite velocity, acceleration and higher time derivatives of disparity. It seems inescapable that the anticipation of the course of sinusoidally varying disparity is based on information concerning the direction in which it is changing, at what rate it is changing, and possibly, what is its acceleration.

The conclusion that it is information concerning the rate of change of disparity which permits the future course of the disparity to be anticipated can be tested by imposing a disparity which changes at a uniform rate. If a clamped disparity is imposed which declines to zero at a uniform rate and then continues to increase in the opposite sense at the same rate, and no anticipation of its course is made, then the response would reverse direction one reaction time after the disparity passes through zero. If, on the other hand, the finite rate of change of the disparity can be utilized to predict the future course, then the turn-about of the response would occur sooner than a reaction time after the disparity passes through zero. Figure 21 shows a typical result of such an experiment. It can be seen that the reversal of the response occurs even before the disparity reaches zero. Allowing for the delay involved in the reaction time, this demonstrates that considerable anticipation of the future course of the disparity has taken place.

## PART III. DISCUSSION

Certain general properties of the observations in Part I can be shown to be compatible with inferences from Part II.

When a sudden change of target vergence is presented to the subject, eye vergence does not begin until a reaction time has elapsed. During the reaction time the disparity is constant and equal to the imposed change of target vergence. The initial velocity of the eye vergence would be expected, therefore, to be that velocity which we have found in Part II to be generated by that disparity. The initial velocities of the responses to sudden changes of target vergence are plotted as open circles in Fig. 22. The black dots are the sustained velocites obtained by clamping the disparity and are replotted from Fig. 13. Although there is little overlap in the ranges of disparity covered by these two sets of observations, it can be seen that the initial velocities from the Part I experiments are a compatible extension of the sustained velocities in the Part II experiments.

It has been shown in Part II that clamped disparities, however small, always generate a sustained eye-vergence movement. As the eye-vergence movement has constant velocity over its complete range, it is reasonable to assume that the velocity which a given disparity evokes is independent of the eye vergence. A stationary state of eye vergence hence cannot coexist with an added disparity. When the subject responds to a sudden

change of target vergence, a state is finally reached in which eye vergence remains stationary. During that state there can, therefore, be no disparity, and this confirms the observation that ultimately the change in eye vergence is equal within the error of measurement to the imposed change in target vergence (see p. 345, also Riggs & Niehl, 1960; Westheimer & Rashbass, 1961).

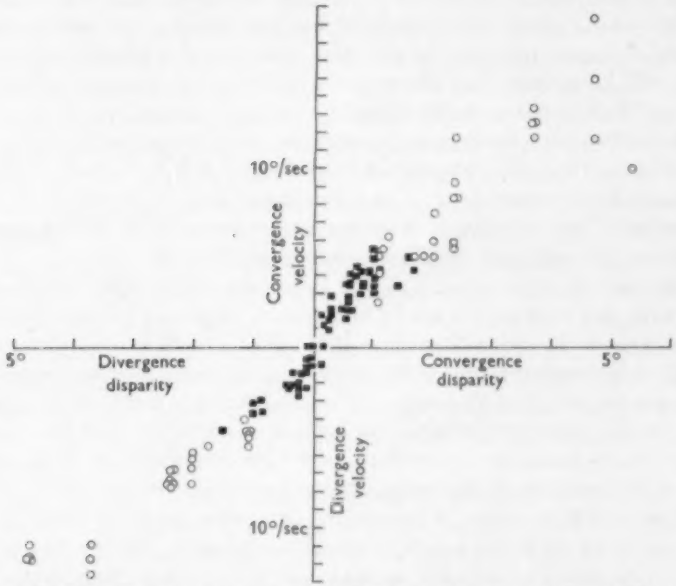


Fig. 22. Velocity of eye vergence plotted against disparity. Open circles are from early phases of eye vergence movements in response to step target vergence stimuli. Black squares are taken from Fig. 13.

In Part II it has been shown that when the disparity is changing, the rate of change influences the responding eye vergence in a way which anticipates the future course of the disparity. Although the quantitative relationship between the rate of change of disparity and its effect on the response is complex and has not been worked out, certain consequences of this effect are discernible in the records shown in Part I. If the rate of change of disparity had no effect, the velocity of the response would be determined only by the magnitude of the disparity one reaction time earlier. In our subjects, with the response parameters determined in Part II, the responses to sudden target vergence changes would be expected to show a large overshoot and a prolonged period of oscillation about the final position. In fact they do not show this. The responses appear to be

those of overdamped or critically damped systems. This may be explained by the effect of rate of change of disparity. As soon as eye vergence starts, the disparity begins to diminish with a finite rate of change. This rate of change will influence the response in a way which tends to reduce the rate at which the disparity is changing. The effect of this will be to reduce or prevent any oscillations about the final position.

The precise effect of the anticipation due to rate of change of disparity depends on the quantitative details of the phenomenon. If a certain degree of rate of change of disparity provokes a moderate corrective effect, the result will be as described above, i.e. a tendency to dampen oscillations that are due to a too rapid attempt to correct disparity. If a certain degree of disparity provokes a greater effect, then it is possible to generate oscillations of velocity. The record from subject E.F.M., shown in Fig. 6, illustrates such oscillations in one direction. The same effect of over-correction of rate of change of disparity is discernible in the response to target-vergence changes with uniform velocity (Fig. 9).

It is clear now that velocity as well as position information is utilized to determine the conjugate tracking oculomotor responses to the movement of images over the retina (Rashbass, 1961). We have shown here that rate of change as well as magnitude of disparity determines eye vergence responses. The estimation of rate of change of disparity might in principle be made in either of two ways: by the difference between the velocity of the movement of the images across the two retinae, or by the rate of change of the difference in the position of the images in the two eyes. The estimation of the speed at which an image is traversing the retina might be made at the retina or more centrally, but any comparison between the two eyes, be it of velocity or position, can only be made centrally. Since the operations of subtraction and differentiation are commutative, i.e. the same result is obtained independently of the sequence in which the operations are performed, this does not restrict the localization of the process of velocity measurement. Whatever the mechanism of the detection of rate of change of disparity and the precise details of its operation, its influence is to stabilize the response to step functions of a system which has appreciable time delay. This stabilization is achieved without unduly prolonging the total response time, and represents an efficient resolution of the conflict between stability and speed of response inherent in any system involving delay in the transfer of information.

## SUMMARY

1. Disjunctive eye movements were recorded in normal subjects while they were presented with target spots, one for each eye, on cathode-ray tubes at a distance of 2 m.

2. Eye-vergence movements in response to suddenly introduced target-vergence changes have a reaction time of about 160 msec, begin with a constant velocity that is a function of stimulus amplitude, and then asymptotically reach their final level with a total movement time of about 800 msec.

3. Responses to momentary changes in target vergence follow each aspect of the stimulus after a reaction time. It is concluded that disjunctive eye movements can be modified during their progress and that information concerning disparity can be assimilated during the reaction time as well as during the movement.

4. Target vergences changing at uniform rate and with simple harmonic motion evoke matching eye-vergence changes after a reaction time. The tracking of higher target-vergence velocities is associated with oscillations. The tracking of high target-vergence frequencies deteriorates after only a few cycles.

5. A method was devised which permits the target vergence to be driven by the eye vergence so that any eye-vergence movements are always accompanied by similar target-vergence movements. When a disparity is now introduced which is then sustained at a constant value, the eye vergence achieves a constant velocity which is a function of the amplitude of the disparity. The constant of proportionality for small disparities is 10°/sec per degree disparity in one subject and 7°/sec per degree disparity in another.

6. The responses to a regular alternation between convergent and divergent disparities, and the shape and amplitude of the eye-vergence changes induced by sinusoidal disparity changes, conform to the pattern of a system the velocity of whose response is a linear function of the degree of disparity.

7. The phase relationship between sine-wave disparity changes and the eye-vergence changes they cause suggests that anticipation of future disparity changes takes place, and an experiment with uniform rate of change of disparity indicates that rate of change of disparity is a factor in such an anticipation.

8. There is general compatibility between the responses to simple controlled disparities and the responses to disparities modified by the movements which the eyes themselves perform.

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# INDEPENDENCE OF CONJUGATE AND DISJUNCTIVE EYE MOVEMENTS

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Conjugate eye movements, in which the two eyes move in a parallel fashion, can be distinguished from disjunctive eye movements, in which the fixation axes of the two eyes move with respect to each other. Recent experiments (Westheimer & Mitchell, 1956; Yarbus, 1957) have demonstrated clearly that the two types of movements can occur simultaneously and seem to be additive. The purpose of this paper is to illustrate by some further experimental results that conjugate and disjunctive eye movements are organized independently.

## METHODS

The subject sat in a dark room and had presented to each eye a small (3 mm) white spot on a cathode-ray tube at 2 m. The spots could be moved independently in a horizontal direction by an externally applied voltage. By a suitable electronic arrangement it was also possible to present independent conjugate and disjunctive target movements of various kinds, including square and sine waves. Target movements were confined to a range of 6–8° centred around the middle of the subject's fusional range.

Eye movements were recorded by a method tending to eliminate, to some considerable extent, artifacts which might arise from lateral translational movements of the eyes. The measure of the positions of the right and left eyes, $\alpha_R$ and $\alpha_L$, which was available in electric form, could be averaged to give the mean position of the two eyes $[\frac{1}{2}(\alpha_R + \alpha_L)]$, and differenced to give the vergence level $(\alpha_L - \alpha_R)$. Details of the method of target presentation and eye movement recording have been described (Rashbass & Westheimer, 1961). Records were obtained on three normal observers. The recordings are estimated to be good to 5′ of arc and have a frequency response flat up to about 30 c/s.

## RESULTS

We have recorded the mean position of the two eyes and the vergence between the eyes while the subjects were presented with targets, one for each eye, under a variety of target movement patterns. If $\alpha_R$ is the angle the fixation line of the right eye makes with the straight-forward line, and

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$\alpha_L$ that of the left eye (using a right-handed co-ordinate system for both eyes), we can define the mean eye position

$$\phi = \tfrac{1}{2}(\alpha_R + \alpha_L)$$

and the vergence

$$\gamma = \alpha_L - \alpha_R.$$

Let $\alpha'_R$, $\alpha'_L$, $\phi'$ and $\gamma'$ be equivalent angles as applied to the targets.

In the first experiment $\phi'$ and $\gamma'$ were each given step changes but at different repetition rates. Figure 1 shows a record taken under these

Fig. 1. Recording of eye vergence (above) and mean lateral eye position (below) when independent step changes are given to target vergence and mean lateral target position. Spikes on records are stimulus artifacts; they indicate the instant a step change was given to the target function.

conditions of $\gamma$ on the upper channel and $\phi$ on the lower one. It is seen that the lateral tracking mechanism for $\phi'$ and the vergence response mechanism for $\gamma'$ each behaves in the manner typical of itself, quite independently of any changes occurring in the other. The response of either system is a normal one (Westheimer, 1954; Rashbass & Westheimer, 1961), whether its step stimulus occurs simultaneously with the stimulus to the other system, during the reaction time of the other system, or during the response of the other system. Since saccadic movements are so short, the question of a convergence stimulus during a saccadic movement is largely academic.

Secondly, $\phi'$ and $\gamma'$ were each given sinusoidal movements at slightly different frequencies. This simulated the movement of a target performing a Lissajous figure in the horizontal plane. The target path, starting as an oblique line from far left to near right, passed through a series of quasi-ellipses of changing eccentricity and obliquity into an oblique line from far right to near left. The path returned in a similar fashion to the original state and the cycle of movements was then repeated. Again there was complete independence of the response mechanism of lateral eye tracking and vergence tracking.

One interesting special case occurs where the convergence component of the targets' motion has a frequency of about 1 c/s. We have shown elsewhere (Rashbass & Westheimer, 1961) that under these conditions the eye vergence follows the target vergence for only a few cycles before

deteriorating into small irregular movements around the mean target vergence. In Fig. 2 is illustrated the response of $\phi$ and $\gamma$ when $\phi'$ and $\gamma'$ are oscillated at frequencies of 1 and 0·66 c/s respectively. Side-to-side tracking proceeds with its normal characteristics, but vergence tracking soon disappears. It follows that the vergence reponses and the smooth pursuit movements are subject to quite different limitations.

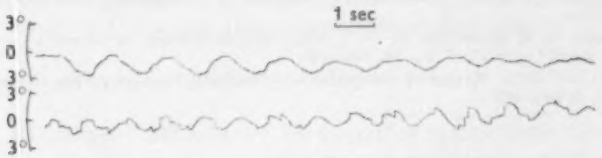


Fig. 2. Recording of eye vergence (above) and mean lateral eye position (below) when independent simple harmonic changes are given to target vergence and mean lateral target position. Note that lateral tracking movement continues unabated while eye vergence, driven at a lower frequency, deteriorates.

It might be argued that vergence and side-to-side eye movements are independent only when these two components are themselves independent simple functions. Finally, we imposed simple harmonic motions of slightly different frequencies on $\alpha'_R$ and $\alpha'_L$. This resulted in a Lissajous figure in the horizontal plane passing through a series of quasi-ellipses from the mid-sagittal horizontal line to a line in a frontal plane. Once again vergence and tracking proceeded independently each in its characteristic manner.

It is concluded that, along whatever path a target moves, the movement is resolved into two components corresponding to mean target position and target vergence, respectively, and that appropriate responses to these components are made by two independent systems.

SUMMARY

1. A spot target was presented to each eye and the two targets were moved to give simultaneous independent stimuli for lateral and for convergence movements. The stimuli were sinusoidal and square-wave forms.

2. The mean eye position follows the mean target position according to the characteristics of visual tracking and simultaneously the eye-vergence response follows the target vergence according to the characteristics of vergence movements.

3. No interaction between the mechanisms was observed; each can accept and respond to stimuli irrespective of whether the other is being stimulated, is within a reaction time, is responding, or is suffering overload.

4. All target movements are resolved into a vergence and a side-to-side component, even when a resolution into right and left eye components would be simpler.

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# THE EFFECT OF NEURONAL REST ON THE OUTPUT OF SYMPATHETIC TRANSMITTER FROM THE SPLEEN

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Brown & Gillespie (1957) studied the output of sympathetic transmitter in the venous blood from the spleen of the cat resulting from stimulation of the splenic nerves. They showed that the output depended upon the frequency of stimulation, being maximal at 30/sec, and falling away at higher and lower frequencies. No noradrenaline could be detected in the venous blood at frequencies lower than 10/sec. The administration of the adrenergic blocking agents dibenamine and dibenyline increased the output at all frequencies below 30/sec. The explanation suggested for these results was that combination with tissue receptors precedes the metabolic removal of liberated noradrenaline; at frequencies below 10/sec the unpoisoned receptor mechanism can remove all the liberated noradrenaline, and so none can be detected in the venous effluent. At frequencies between 10 and 30/sec increasing amounts overflow into the circulation because the receptor mechanism is swamped. The administration of a blocking agent prevents the uptake of transmitter by the tissue, and the noradrenaline then appearing in the venous blood gives a measure of the amount liberated by the nerve endings. It is therefore possible by measuring the amount of transmitter in the venous blood, before and after a blocking agent is given, to determine, for any frequency of stimulation, the amount of transmitter liberated, the amount taken up by the tissue, and the amount normally overflowing.

Some experiments made for another purpose suggested that previous activity might modify the amount of the transmitter overflowing when the nerves were stimulated. It had been found, for instance, that a first group of stimuli at 30/sec given to the nerve after a rest of 1–1½ hr yielded 450 pg/stimulus, whereas a second, given 10 min later, produced an overflow of 1025 pg/stimulus (mean of 5 observations). These experiments led us to think that interruption of the normal constant centrifugal discharge of the sympathetic neurones might alter the peripheral processes of liberation

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and uptake of transmitter and yield information on the changes underlying the well-known sensitizing effect of decentralization. The innervation of the spleen by post-ganglionic fibres, with a cell station in the coeliac ganglia, provided an opportunity of studying the effect of resting the neurones by cutting the splanchnic nerves and so decentralizing them. Rest in this way has been found to modify the overflow of transmitter into the venous blood, but the picture is complicated by even greater changes in the amount of transmitter liberated by the nerve endings and in the amount of liberated transmitter taken up by the effector cells of the spleen. Preliminary accounts of some of this work have already been published (Brown, Davies & Ferry, 1959; Brown, 1960).

## METHODS

Cats were used for all the experiments on the spleen. Before the operation to decentralize the spleen, the animal was given 1 mg atropine sulphate by subcutaneous injection and then anaesthetized with ethyl chloride and ether. The left splanchnic major nerve and usually one or more minor nerves were divided through an incision in the left flank, with strict aseptic precautions. In the subsequent experiment, between 19 hr and 6 days later, the cat was anaesthetized with ethyl chloride and ether and then chloralose 80 mg/kg was given intravenously. The abdomen was opened in the mid line and the splenic nerves were dissected off the splenic artery and tied. The arrangements for nerve stimulation, for collection of blood and for the assay of its noradrenaline content on the blood pressure of the completely pithed rat were identical with those described by Brown & Gillespie (1957).

Section of the left splanchnic major nerve almost completely decentralizes the post-ganglionic neurones to the spleen. In four experiments stimulation of the left splanchnic major nerve at 30/sec gave a mean output of noradrenaline of 1014 pg/stimulus. This figure is within the normal range of output when the splenic nerves are stimulated at this frequency. Stimulation of the right splanchnic major nerve in the same animals gave a mean output of 224 pg/stimulus. Hence the left splanchnic provides 80% of the pre-ganglionic fibres supplying the spleen.

In all experiments division of the splanchnic nerve was confirmed by post-mortem examination.

The adrenergic blocking agents used in the investigation were: dibenyline, dibenzyline (*N*-phenoxy*iso*-propyl-*N*-benzyl-$\beta$-chloroethylamine) 10 mg/kg by intravenous injection; hydergine (Sandoz; a preparation of dihydro-ergocornine, dihydro-ergocristine and dihydro-ergokryptine) 0·5 mg/kg by intravenous injection; phentolamine (Rogitine, Ciba; (2-*N*-p-tolyl-*N*-m-hydroxyphenyl-aminomethyl)-imidazoline methanesulphonate) was used in one experiment only.

## RESULTS

*Overflow of transmitter in normal animals.* All the experiments described in this paper have been made with two frequencies of stimulation only, 30/sec which gives maximal overflow, and 10/sec which gives the least detectable overflow of noradrenaline in the venous blood. The figures we have obtained differ a little from those published by Brown & Gillespie, but have not substantially changed the characteristic output–frequency curve (Fig. 1 and Table 1).

*The effect of adrenergic blocking agents in normal animals.* The effect of the blocking agents dibenamine and dibenyline on the output of noradrenaline was investigated by Brown & Gillespie, who gave them 24 hr before an experiment and report only two experiments in which dibenyline was given in the course of an acute experiment. We have now accumulated a number of results in which dibenyline or other blocking agents were given either immediately after adrenalectomy or immediately after the first stimulation sample had been collected. In both instances half to one hour was allowed to elapse before sampling was continued.

TABLE 1. Overflow of noradrenaline from the spleen on stimulation of the splenic nerves with 200 shocks

| | Brown & Gillespie (1957) | | | Recent results | | |
|---|---|---|---|---|---|---|
| Frequency | Mean | S.E. of mean | No. of expts. | Mean | S.E. of mean | No. of expts. |
| 10/sec | 166 | 20 | 14 | 169 | 28 | 25 |
| 30/sec | 985 | 115 | 23 | 841 | 61 | 36 |

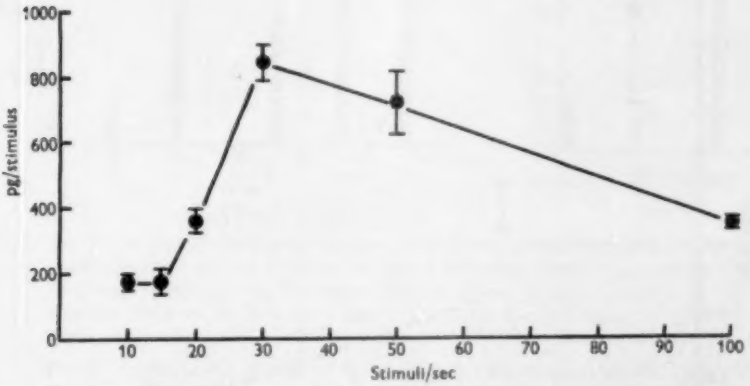


Fig. 1. Relation between overflow of noradrenaline and frequency of stimulation of the splenic nerves in normal animals. Each point shows the mean and S.E. of mean of several experiments. Points other than at 10 and 30/sec are from Brown & Gillespie (1957). For further details see Table 1.

At 30/sec there is a small, just significant increase, but at 10/sec the output in the venous blood is increased by a factor of nearly ten to give the high figure of 1710 pg/stimulus (Fig. 2, Table 2). This output after blocking agents we have taken to represent the liberation of noradrenaline by the nerve endings.

We have carried out experiments with two other adrenergic blocking agents, hydergine and phentolamine. The results of one experiment with

hydergine are shown in Fig. 3. In this experiment hydergine 0·5 mg/kg was given between groups of 200 stimuli at 10/sec and 30/sec. The output at 10/sec is raised to that of 30/sec. This pattern of output is what we expect when dibenyline is used. In our more recent experiments we have used hydergine regularly, as it has the advantage over dibenyline of quickly producing a nearly complete block to nerve stimulation. A sample

TABLE 2. The liberation of noradrenaline from the spleen on stimulation of the splenic nerves after dibenyline or hydergine

| | Brown & Gillespie (1957) | | Recent results | | |
|---|---|---|---|---|---|
| Frequency | Mean | No. of expts. | Mean | S.E. of mean | No. of expts. |
| 10/sec | 1220 | 10 | 1710 | 280 | 8 |
| 30/sec | 778 | 5 | 1060 | 132 | 14 |

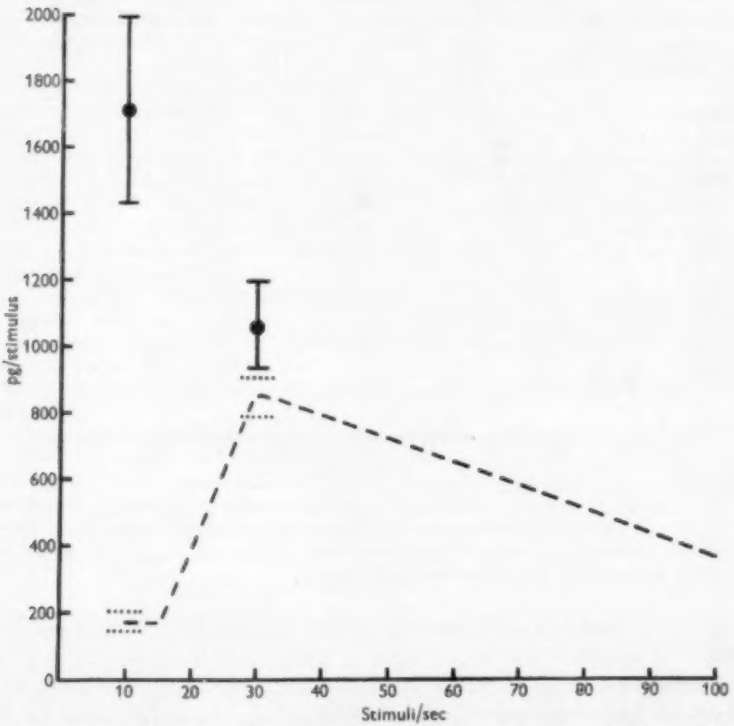


Fig. 2. Effect of adrenergic blocking agents on output of noradrenaline from the spleen with stimulation of the splenic nerves at 10 and 30/sec, indicated by the two single points. The interrupted line shows the normal overflow curve. For further details see Table 2.

can therefore be taken 10 min after administration of hydergine, whereas 30 min must be left between administering dibenyline and taking the next sample, during which time the preparation may deteriorate. Bilateral vagotomy is necessary before the administration of hydergine, to abolish the vagal inhibition of the heart otherwise produced by the drug.

In one experiment we used phentolamine intravenously in a dose of 2·8 mg/kg. The results of this experiment are shown in Fig. 4, and it can be seen that the drug caused an elevation of the output at 10/sec in a way similar to the other adrenergic blocking agents.

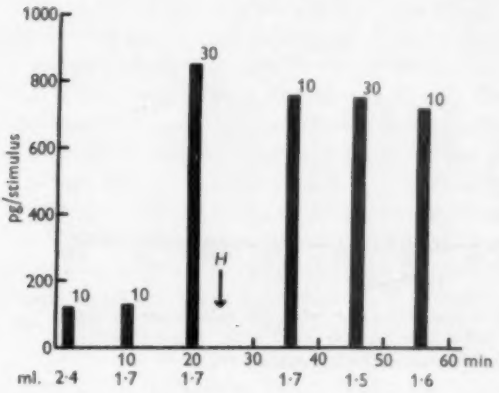


Fig. 3

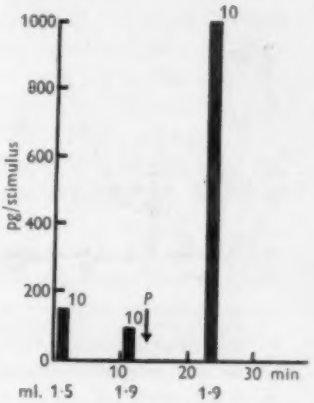


Fig. 4

Fig. 3. Cat 3·5 kg. Output of noradrenaline from the spleen in response to groups of 200 stimuli to the splenic nerve. The figures at the top of each block indicate the frequency of stimulation. The plasma volume of each sample is given below the abscissa. Between the third and fourth samples hydergine 0·5 mg/kg was given by intravenous injection.

Fig. 4. Drawn as Fig. 3. Cat 1·7 kg. Output of noradrenaline after 200 stimuli at 10/sec. Between the second and third samples phentolamine 2·8 mg/kg was given intravenously.

### *The effect of neuronal rest*

*Effect of rest on the overflow at* 30/*sec.* When the abdomen was opened 19 hr to 6 days after cutting one or both major splanchnic nerves the spleen was seen to be large and engorged with blood. Stimulation of the post-ganglionic trunk caused a vigorous contraction and expulsion of blood with a high cell volume. When the overflow of noradrenaline with stimulation at 30/sec was determined, it was found to be significantly lower than the normal. In normal cats the overflow of noradrenaline from the spleen is 841 pg/stimulus (S.E. 61); in the 29 rested animals it was 394 pg/stimulus

(S.E. 30). This is illustrated in Fig. 5. It appears that section of the left splanchnic major nerve is sufficient to produce these effects. In 5 of the 29 experiments the splanchnic major nerves on both sides had been cut; in other experiments the left splanchnic minor and minimus had been cut as well as the left splanchnic major. There was no difference in the results attributable to section of nerves in addition to the left splanchnic major.

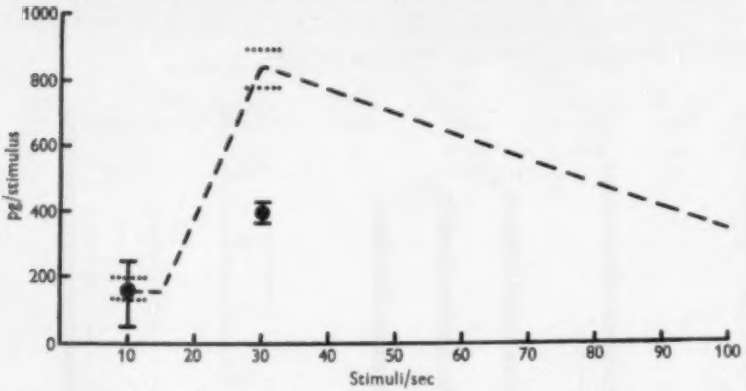


Fig. 5. Overflow of noradrenaline from the spleen when the splenic nerves were stimulated at 10/sec and 30/sec in the decentralized preparation. Interrupted line shows the normal overflow curve. For further details see text.

*Effect of rest on the overflow at* 10/*sec.* Having found that rest caused a decrease in the overflow of noradrenaline when the nerves were stimulated at 30/sec, we expected that the overflow at 10/sec would be very small indeed. In six experiments, however, the initial overflow was 148 pg/stimulus (S.E. 100). This point is also shown in Fig. 5, superimposed on the normal overflow curve. It can be seen that there is no significant difference between the normal and the rested overflow at 10/sec.

The reduction in output of transmitter in the rested preparation is not due to any general effects of the previous operation nor to treatment of the animal with atropine or anaesthetics. In one animal, for instance, the left splanchnic nerve was not cut because of haemorrhage, and 24 hr later the output at 30/sec was 1000 pg/stimulus before, and 1100 pg/stimulus 1 hr after the administration of dibenyline, figures well within the normal range. In another animal the right splanchnic was divided and the left was sought, but not found. Four days later the output at 30/sec was 600 pg/stimulus. After 1000 stimuli at 30/sec the output remained at 600 pg/stimulus. This behaviour is characteristic of a normal unrested preparation with an output somewhat below the mean.

The diminution from the normal values of the overflow at 30/sec in the rested preparation could have been due either to diminished liberation of transmitter or to increased uptake by the tissue. The fact that there was no change in the overflow at 10/sec gave no clue to the cause of the reduction at 30/sec, and the only means of testing the point was to use blocking agents and thereby gain some information on the effect of rest on the amount of transmitter liberated.

*Effect of adrenergic blocking agents on the output after rest.* The administration of a blocking agent to a rested preparation showed that the liberation of noradrenaline after rest is greatly in excess of the normal. In 12 experiments the splenic nerve was tied as soon as the animal was anaesthetized with chloralose, and a blocking agent was given at a suitable time after adrenalectomy. In 7 cats the first stimulation was at 10/sec and the liberation was 3060 pg/stimulus (S.E. 465). In the other 5 animals the first stimulation was at 30/sec and the liberation was 1800 pg/stimulus (S.E. 206). The normal values for the liberation of noradrenaline are 1710 pg/stimulus at 10/sec and 1060 pg/stimulus at 30/sec. These points, together with the normal overflow curve, are shown in Fig. 6.

We conclude from these experiments that resting the post-ganglionic neurone leads to an increase in the amount of noradrenaline liberated per stimulus and also to an increase in the capacity of the tissue to take up the transmitter. At 30/sec the increase in liberation is less than the increase in uptake, and therefore the overflow falls. At 10/sec the increases in liberation and in uptake are about the same and the overflow, in consequence, is unchanged.

*The background pressor activity of plasma.* Furchgott (1959) has suggested that the elevation of the output from the spleen after dibenyline might be due to the release by the drug of noradrenaline from the nerve endings. During our experiments we have taken a number of samples of splenic venous blood in the absence of stimulation of the splenic nerves and assayed this background pressor activity in terms of noradrenaline. The initial venous control sample at the beginning of every experiment usually contains some substance with a pressor activity equivalent to about 10–20 ng noradrenaline/ml. The active substance in this sample is probably not noradrenaline, as it has a slow, long-lasting effect on the blood pressure of the pithed rat. During an experiment in the absence of blocking agents the background pressor activity gradually increases to about 30 ng/ml. and resembles that of catecholamines. We assume this additional activity to be produced by sympathetic activity in a deteriorating preparation which is being gradually exsanguinated during the experiment (cf. Brown, Davies & Gillespie, 1958).

If a blocking agent is administered, the background activity rises in a

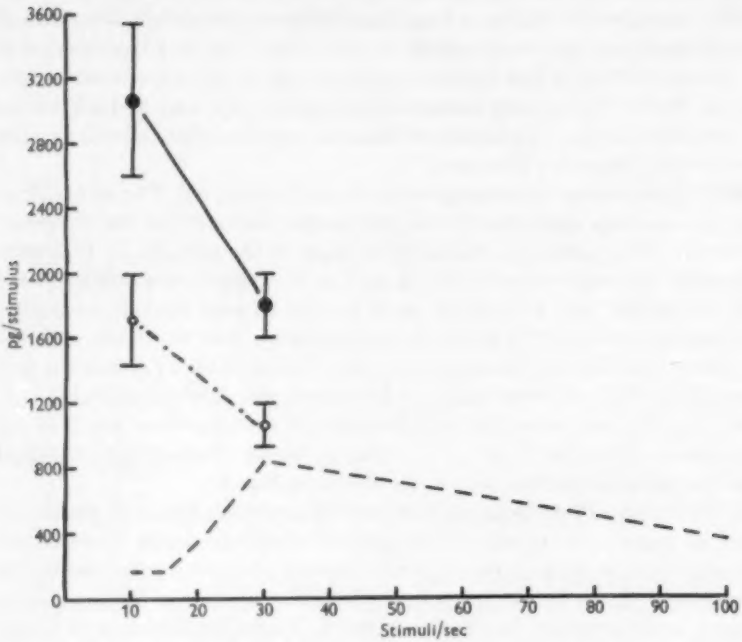


Fig. 6. Liberation of noradrenaline (mean and S.E. of mean) in the decentralized preparation (●), together with the liberation in the normal (○) and the normal overflow curve. For further details see text.

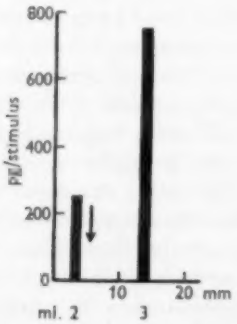


Fig. 7. Cat 2·7 kg. Left splanchnic nerves cut 24 hr previously. Output of noradrenaline after 200 stimuli at 30/sec to the splenic nerve. Between the two samples 1000 stimuli at 30/sec were given at the arrow.

similar way. After the administration of dibenyline an interval of at least 30 min was allowed to elapse before continuation of the experiment. During this time the background activity rose again to about 30 ng/ml. The use of hydergine allowed sampling to be resumed 10 min after administration of the drug, and the rise of background activity was usually absent or very small. Venous control samples taken some time after the administration of this drug showed a rise similar to that occurring after dibenyline. The rise in background pressor activity at various times after administering blocking agents is thus closely similar to the rise in their absence. We have therefore no reason to suppose that release of noradrenaline by the blocking agents is a significant factor in these experiments.

*Effect of a conditioning train of stimuli on the overflow after rest.* It seemed to us that, if the effects described above were due to the cessation of the normal discharge in the post-ganglionic pathways, a conditioning train of stimuli ought to return the transmission mechanism towards the normal. This proved to be the case, and we have found that the low initial overflow at 30/sec can be raised by further stimulation. The application of the standard sampling group of 200 stimuli alone has a definite effect, and the second sample always contains more noradrenaline than the initial sample, an effect referred to in the introduction to this paper. A more dramatic way of elevating the overflow is to give a conditioning train of 1000 stimuli at 30/sec. In five experiments the mean initial overflow at 30/sec was 381 pg/stimulus. After a train of 1000 stimuli at the same frequency the second mean overflow was 936/stimulus. One of these experiments is illustrated in Fig. 7.

It appears from these experiments that the effects of rest on the overflow at 30/sec can be removed by a period of stimulation of the nerves. The change could be due either to an increase in the liberation or to a reduction of uptake to within normal limits. This was tested by treating rested preparations with dibenyline *ab initio* and measuring the overflow with 200 stimuli, before and after 1000 stimuli at 30/sec. The results we obtained were not entirely conclusive, but in the few satisfactory experiments there was a small reduction in the liberation. It would appear therefore that the chief effect of 1000 stimuli is a reduction in uptake by the tissues.

*Experiments with ganglionic blocking agents.* We thought it might be possible to rest the post-ganglionic nerves to the spleen by the use of ganglionic blocking agents. Two drugs were tried, pentolinium tartrate (Ansolysen; May and Baker), and chlorisondamine (Ecolid; Ciba). These are reported to block ganglia maximally for 4 and 8 hr respectively. We injected them subcutaneously in doses of 3 mg/kg and used the nictitating membrane as an indicator of the state of block. With chlorisondamine the

nictitating membranes were relaxed across the eyes within 10 min of the injection and remained so for about 6 hr. After this time the block gradually wore off, and we could therefore not keep a cat blocked overnight. We found that the effect of a subcutaneous injection could be prolonged up to 16 hr by injecting chlorisondamine (4 mg/kg) in aluminium stearate in arachis oil.

In four experiments in which the block had been effective for 20–24 hr there was a clear reduction in the initial output of transmitter in response to 200 stimuli at 30/sec, the mean value being 270 pg/stimulus, and in all these experiments the outputs could be increased either by a conditioning train of stimuli at 30/sec or by dibenyline. In experiments in which the block was in operation for under 4 hr there was no evidence of any lowering of the initial output, and conditioning stimulation did not cause a significant increase in output. With periods of block between 4 and 20 hr the results were equivocal, the effect sometimes being evident and at other times absent, with no obvious relation to the duration of the block.

*Effect of injecting noradrenaline into the spleen.* Resting the postganglionic neurones to the spleen reduces the initial overflow of noradrenaline when the nerves are stimulated at 30/sec. We have interpreted this reduction as being mainly due to a temporary increase in the ability of the tissue to take up the transmitter. This increased avidity of the tissue can be returned towards normal by a conditioning train of stimuli. Theoretically a close-arterial injection of noradrenaline into the spleen should satisfy this avidity and increase the overflow of noradrenaline on subsequent stimulation of the splenic nerve.

We therefore measured the initial overflow of transmitter at 30/sec and then injected noradrenaline into the spleen through a cannula in the hepatic artery. Ten minutes later the usual 200 stimuli at 30/sec were given and the overflow of noradrenaline determined. Ten micrograms of noradrenaline in 1 ml. of saline was used as the standard injection, since this amount caused approximately the same degree of contraction of the spleen as stimulation of the splenic nerves. These experiments proved rather unsatisfactory; the overflow in the second and third samples was greater than in the initial sample, but the results were almost identical with the controls in which the injection of noradrenaline was omitted. A possible cause of our failure to raise the reduced output is that the injection of such a large quantity of noradrenaline into the spleen caused a prolonged contraction, presumably through some of the noradrenaline being trapped in the closed vessels, and the blood flow, in consequence, was poor during the collection of the first, crucial sample after the injection. Smaller doses of noradrenaline had little effect on the spleen or on the overflow with a subsequent stimulation.

*The nature of the transmitter liberated after rest.* The transmitter liberated from normal splenic nerve endings is mainly noradrenaline (Peart, 1949; Mann & West, 1952; Brown & Gillespie, 1957), the adrenaline content being seldom more than 10% (Mirkin & Bonnycastle, 1954). It is conceivable that in the rested neurones a greater proportion of adrenaline might be present in sympathin released on excitation. As the pithed rat is less sensitive to adrenaline than to noradrenaline, a change in the ratio of the two substances in the venous effluent might explain some of our results. In one experiment in a cat 3 days after section of the left splanchnic nerves hydergine was administered and the splenic nerve was stimulated at a frequency of 30/sec. The plasma was treated so as to separate catecholamines (Vogt, 1952) and adrenaline was assayed on the carbachol-stimulated rat uterus (Gaddum & Lembeck, 1949), the noradrenaline being estimated in the usual way. The results showed that the sympathin liberated from rested neurones contained about 11% adrenaline. We conclude that resting the neurones does not alter the nature of the liberated transmitter.

*The pattern of outflow of transmitter.* The decentralized spleen contracts more vigorously in response to stimulation of the splenic nerves than does the control. It is possible that this might alter the time relations of outflow of transmitter, and the standard sampling time—the period of stimulation + 20 sec—might not allow collection from the rested preparation of the same proportion of total outflow as in the normal. We have found that the outflow pattern of the rested preparation does not differ significantly from the normal, and that 85–95% of the total outflow appears within the standard sampling time.

*Effect of duration of rest.* It has been shown by Govaerts (1935, 1939) and by McLennan & Pascoe (1954) that spontaneous activity of decentralized ganglion cells begins after 3 days and is fully developed 7 days after preganglionic section. The following figures show the number of experiments performed with different periods of rest:

| Duration of rest in days | ... | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Number of experiments | ... | 15 | 3 | 6 | 2 | 1 | 2 |

The decrease in overflow at 30/sec was fully developed after 24 hr rest; no difference could be detected with durations of rest up to 6 days.

## DISCUSSION

### *The normal preparation*

The experiments that have been recorded in this paper have confirmed and extended those of Brown & Gillespie (1957) and have strengthened the evidence on which they based their conclusion that noradrenaline liberated from nerve endings is inactivated after combination with tissue receptors.

Inhibitors both of aminoxidase and of *o*-methyltransferase (Bacq, Brown & Ferry, 1960) are without effect on the overflow of the liberated transmitter, and a variety of substances with the common property of blocking adrenergic receptors have identical and dramatic effects on the overflow of noradrenaline. The hypothesis put forward by Furchgott (1959) that dibenyline might owe its effect not to its blocking action but to an inhibition of some unknown enzyme system would appear to be untenable, unless the enzyme is identical with the receptive substance for noradrenaline.

TABLE 3. Rates of liberation and uptake in normal and rested spleen; rates are expressed in ng/sec

| | Normal | | | Rested | | |
|---|---|---|---|---|---|---|
| Frequency | Liberation (*a*) | Uptake (*b*) | *b*/*a* | Liberation (*a*) | Uptake (*b*) | *b*/*a* |
| 10/sec | 17 | 15 | 0·88 | 31 | 29 | 0·94 |
| 30/sec | 32 | 7 | 0·22 | 54 | 42 | 0·78 |

We have assumed that the output of noradrenaline in the absence of a blocking agent represents the overflow of transmitter, and that the output in the presence of a blocking agent represents the amount of transmitter liberated from the nerve endings. This cannot be strictly true because none of the blocking agents that we have used has produced a complete block to nerve stimulation, although, as is well known, the block to injected noradrenaline is total. We have no means of determining what proportion of the amount liberated is still taken up by the receptors, but it is likely to be small since Nickerson (1956) has shown that a maximal contraction of smooth muscle to histamine can be produced by a dose occupying not more than 1 % of the receptors.

From the figures of liberation and overflow the amount and rate of uptake of noradrenaline by the receptive substance can be calculated (Table 3). It is clear that at 30/sec the uptake is depressed, although the rate of liberation is twice what it is at 10/sec. This state of affairs is reminiscent of the desensitization of cholinergic receptors by the iontophoretic application of acetylcholine (Kutz & Thesleff, 1957; Axelsson & Thesleff, 1958) or by repetitive stimulation of the motor nerve (Thesleff, 1959), and their suggestion that excess transmitter may inactivate the receptor fits well with our findings.

Brown & Gillespie (1957) thought that the liberation of noradrenaline per stimulus was probably constant from low frequencies up to 30/sec. Our more complete results with blocking agents show that the liberation per stimulus at 10/sec is considerably higher than at 30/sec. Unfortunately our experimental technique has not allowed accurate measurements at frequencies below 10/sec, but it would appear that from 10/sec to 30/sec

and beyond there is a steady decline of liberation. This is probably due to prejunctional failure, since the trunks we were stimulating were C fibres of small diameter, and even in the frog's skeletal muscle–nerve preparation prejunctional failure occurs when frequencies of stimulation much above 10/sec are used (Krnjević & Miledi, 1958, 1959).

*The effect of rest*

Decentralization for 24 hr of the post-ganglionic neurones supplying the spleen has been shown to bring about both an increased liberation of transmitter when the nerves are exposed to a short train of stimuli and an increased capacity of the receptive mechanism to take up the liberated transmitter. The peripheral sensitization both to nerve stimulation and to injected drugs does not reach its maximum until rather later (cf. Cannon & Rosenblueth, 1949), but we have avoided using longer periods than 6 days because of the possibility of the early re-innervation of the coeliac ganglion from adjacent cholinergic neurones.

The majority of our experiments have been done on animals 24 hr after splanchnic section, but we could find no evidence of a diminution of the effect of decentralization in animals examined up to 6 days after nerve section. It would appear, therefore, that the spontaneous discharge of the ganglion cells, to which we have already referred, has had no significant effect. We have shown that 1000 stimuli at 30/sec can abolish the effect of rest, but some experiments at present in progress suggest that stimulation at lower frequencies may be quite ineffective. It is possible, therefore, that removal of the effect of rest requires the presentation to the uptake mechanism of noradrenaline at a high rate. If this is so, it would explain the absence of effect of the irregular and infrequent spontaneous discharge and possibly also our failure to raise the rested overflow with injections of noradrenaline.

The doubling of the uptake of transmitter that we have observed suggests that the elimination of centrifugal impulses to the spleen brings about, in the smooth muscle cells, an increase in the area of receptive substance capable of taking up transmitter, just as denervation evokes a spreading of the area sensitive to acetylcholine in skeletal muscle (Axelsson & Thesleff, 1959; Miledi, 1960). A similar increase in receptor area can be produced in skeletal muscle by blocking conduction with botulinum toxin (Thesleff, 1960), and smooth muscle is sensitized to noradrenaline by reducing its noradrenaline content with reserpine (Burn & Rand, 1958), or by preventing the liberation of transmitter with bretylium (Green, 1960). There is clear evidence, however, that decentralization of the spleen for 3–14 days does not alter either its catecholamine content or the adrenaline: noradrenaline ratio (Rehn, 1958). The great increase in the amount of

transmitter liberated when the nerves of a rested preparation are eventually stimulated makes it probable that neuronal rest produces an accumulation of transmitter at the nerve endings, and it is possible to envisage a neuromuscular junctional apparatus, composed of nerve endings and receptive substance in close apposition, with a constant noradrenaline content, the partition between ending and receptor being controlled by the number and frequency of nerve impulses reaching the ending. Such a conception implies that when an amount of transmitter is liberated it combines with a given area of receptive substance or given number of receptors, activates the tissue and then remains for an appreciable time in combination with the receptors, which, as a result, are rendered inaccessible to further doses of transmitter. The easy reversibility of the effects of rest by 1000 stimuli at 30/sec is compatible with this view and particularly so since the effects of stimulation are of relatively long duration. A single train of 1000 stimuli abolishes the effect of rest, and if this is reinforced at 10 min intervals by groups of 200 stimuli for testing purposes, the preparation in an experiment lasting some hours never reverts to the rested state. We have some evidence that a rest of 1 hr can produce a reduction in transmitter overflow, but at least 12 hr must elapse before the reduction is beyond question. This implies that the processes responsible for removal of transmitter from the receptors take some hours to be effective and yet, during this period, the cells with some receptors occupied can still respond to further liberations by the nerve. These observations are compatible with Paton's (1961) suggestion that it is the 'rate of uptake of agonist' by receptors which determines activity of the effector cell and not the occupation *per se* of receptive sites.

SUMMARY

1. The output of noradrenaline from the spleen of the cat under chloralose in response to maximal excitation of the splenic nerves has been studied further.

2. The observation of Brown & Gillespie (1957) that adrenergic blocking agents (phenoxybenzamine) elevated the output of transmitter has been confirmed and extended to other blocking agents, hydergine and phentolamine. The output at a frequency of stimulation of 10/sec is increased by a factor of 10 and that at 30/sec is just significantly increased.

3. It is assumed that the output after blocking agents represents the amount of transmitter liberated and the output without a blocking agent represents the overflow. From these two values the tissue uptake can be determined.

4. At frequencies of stimulation of 10/sec 90% of the liberated transmitter is taken up by the tissue. At 30/sec the rate of liberation is twice that at 10/sec but only 28% of the liberated transmitter is removed by the tissue.

5. Degenerative section of the left splanchnic nerve decentralizes 80% of the post-ganglionic neurones supplying the spleen.

6. When the splenic nerve is stimulated at 30/sec 19 hr to 6 days after splanchnic section, the overflow of noradrenaline is reduced by half. At 10/sec the overflow is unchanged.

7. The liberation of transmitter (measured after blocking agents) is doubled at 10/sec and nearly doubled at 30/sec. The depression of overflow therefore is due to a great increase in the uptake of transmitter by the tissue.

8. The depressed overflow at 30/sec can be restored by stimulation of the splenic nerve with a train of 1000 stimuli.

9. The effects of splanchnic section can be reproduced with ganglionic blocking agents.

10. Neuronal rest, therefore, appears to cause an accumulation of transmitter at the nerve endings and to give rise to a greatly increased capacity of the innervated tissues to take up liberated transmitter.

This work was carried out during the tenure of M.R.C. scholarships by two of us (B.N.D. and C.B.F.) and of an M.R.C. grant for Scientific Assistance (G.L.B.). We wish to thank Mrs C. Edwards and Mrs H. Parsons for technical assistance. We are grateful to Mr W. G. Bradley for the assays of plasma for adrenaline, and to Dr T. D. Whittet for the preparations of ganglion blocking agents.

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## A simple bath for nerve muscle experiments in the class-room

By R. COLLIN and J. FIRMAN. *Department of Physiology, University of Western Australia*

In a hot dry climate it has been found that the amphibian gastrocnemius sciatic nerve preparation rapidly dries out if suspended in air. The bath illustrated is offered as a simple and cheap solution to this problem.

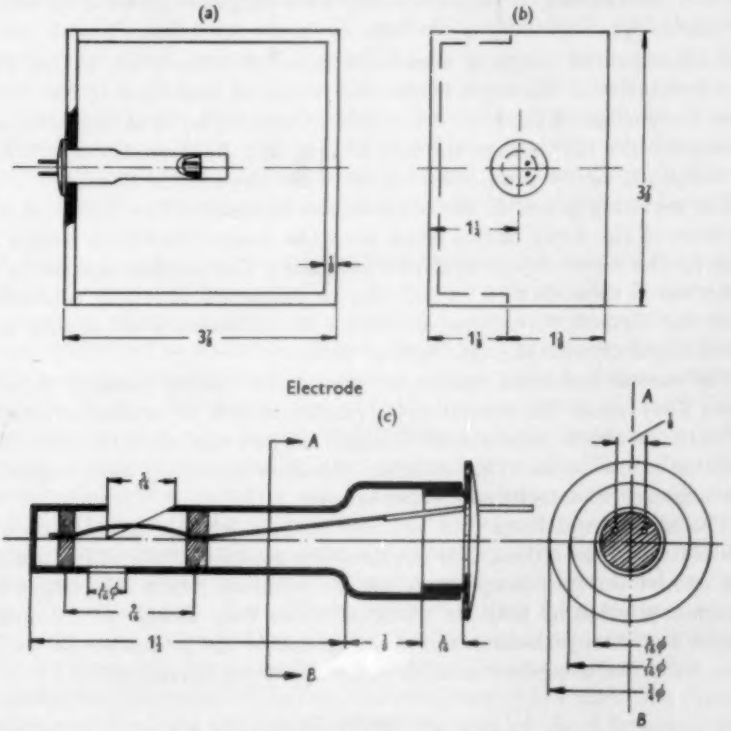


Fig. 1. Nerve muscle bath for use in hot climates. (*a*) Plan; (*b*) side elevation; (*c*) details of the stainless-steel electrode assembly.

The bath is made of $\frac{1}{8}$ in. (3 mm) polythene sheet and measures 10 cm × 10 cm × 3 cm. Electrodes are made of stainless-steel wire and are mounted in the plunger of a disposable 2 ml. plastic syringe. The electrodes should be curved to drain off any excess of Ringer's solution. The wires from the stimulator are crimped into hypodermic needles of suitable gauge, which are then pressed on to the stainless-steel electrodes. The electrode assembly is held in a hole through an extension of one of the walls so that it lies horizontally 3·2 cm above the floor of the bath.

The muscle is dissected together with the femur. The preparation is mounted in the bath by means of a Palmer muscle clamp which grips the end of the femur. The nerve is placed on the electrodes only when it is to be stimulated.

## Diffraction images in the compound eye

By E. T. Burtt and W. T. Catton. *Departments of Zoology and Physiology, King's College, University of Durham, Newcastle upon Tyne*, 1

A succession of images is observable in an eye slice of the locust. The first is that due to the single lenslet and is formed at a depth of 200–250 $\mu$ from the surface of the eye. Second, third and higher order images arise as interference effects from the first images, but the basement membrane at a depth of *ca.* 600 $\mu$ excludes higher orders beyond the third.

The resolving power of the single lenslet is measured by adjusting the distance of the array of fine wires until the image behind the lenslet as seen by the 4 mm objective is just indistinct. The angular separation of the wires is then about 1° which can be compared with 1·2° calculated from the formula $\theta$ (radians) = $1{\cdot}22\,\lambda/d$ for an aperture ($d$) of 32 $\mu$ and wave-length ($\lambda$) of 0·56 $\mu$ (cf. Barlow, 1952).

The second and third images are seen in the deeper parts of the eye slice. They allow the resolution of smaller angles of separation of the wires in the object; thus the third image allows a resolution of 0·35°–0·4°.

Silver preparations of the retinula cells show that they have a spacing close enough to detect such deeper images.

The second and third (but not the first) images are affected by the orientation of the object. For one complete rotation of the object around the axis of the microscope there are six positions where the images are clearest, alternating with six positions where they disappear. It is suggested that this phenomenon may be related to the preference shown by bees for radial as against parallel striped patterns (Hertz, 1931).

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## A simple blood flowmeter

By A. L. HAIGH and P. SANDLAND. *Department of Physiology, University of Birmingham*

In experiments previously reported (Cumming, Haigh, Harries & Nutt, 1961) total gastric blood flows varying from 8 to 60 ml./min were obtained in the dog. Most of the flow rates are too large to be measured with a drop counter. A flowmeter to deal with blood flows of this order has been devised which is of simple construction, records with inexpensive equipment on a kymograph, and is easy to use.

Blood enters the polythene inflow cannula and passes in plastic tubing to a polythene T-junction, the side arm of which forms a pressure manometer. The bubble introducer consists of a 2 ml. automatic pipetting syringe, and is joined to the side arm of a second T-junction placed immediately after the first. The volume of bubble used is 0·2 ml. The bubble introducer is followed by a short length of glass tubing which passes through the photo-transistor unit to the bubble trap. Glass is necessary to obtain sufficient change of light intensity when the bubble passes between the light source and the photo-transistor. The bubble trap has two outlets, the lower one through which blood is returned to the outflow cannula, and the other, at the top of the trap, from which air can be removed periodically. Total volume is 45 ml. and that of the glass flow tube 2·0 ml. The apparatus is treated throughout with silicone and primed with saline before use.

A simple electrical device times the passage of air bubbles from the syringe to the photo-transistor. The first multivibrator (Fig. 1) operates at intervals, which can be varied according to flow rate from 7 to 30 sec, a relay connected to a solenoid. When the solenoid is energized, the core, which is in contact with the spring loaded syringe plunger, moves forward depressing it, thus introducing an air bubble into the stream of blood. This relay also triggers the second multivibrator giving impulses (100 V, 20 msec) which operate the 'impulse' electromagnet of a Thorp Impulse Counter raising the recording arm. Impulse frequency can be adjusted (1, 3 or 6 c/s) according to flow rate to obtain convenient heights of record. Impulses continue to raise the recording arm until the bubble passes the photo-transistor, operating the other relay connected to the 'timer' electromagnet of the Thorp counter, which trips the recording arm to zero. At the same time this second relay resets the solenoid relay ready for the next cycle to be triggered off by the first multivibrator.

The height of the record obtained is the reciprocal of the blood flow which is calculated from the calibration graph. Records of blood flow may be obtained from 7 to 30 sec intervals, depending on the rate of flow.

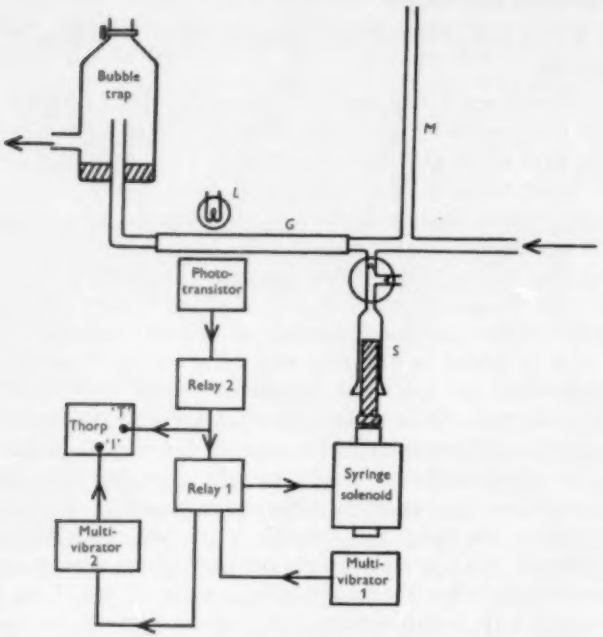


Fig. 1. Diagrammatic plan of flowmeter. *M*, Manometer; *S*, automatic pipetting syringe; *G*, glass flow tube; *L*, 6·5 V, 0·3 A pilot lamp.

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## Isoenzymes of human alkaline phosphatase and lactic dehydrogenase

By A. W. HODSON, A. L. LATNER, LAUREEN RAINE and A. W. SKILLEN.
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Several enzymes occur in a number of different forms within individual tissues (Markert & Møller, 1959). These enzyme components have closely similar biochemical activities, but differ in relation to their pH maxima, their behaviour during starch-gel electrophoresis and in certain immunochemical reactions. These components are now known as isozymes or isoenzymes. The isoenzymes which have been most completely studied are those of certain dehydrogenases with particular reference to lactic

acid dehydrogenase. It has been shown that in disease of a tissue its lactic-acid dehydrogenase isoenzyme pattern becomes imposed on the normal pattern of serum (Wróblewski & Gregory, 1961). This shows great promise in relation to diagnosis.

Studies have been carried out in this laboratory with alkaline phosphatase and lactic acid dehydrogenase using staining techniques to demonstrate the zones of activity resulting from starch-gel electrophoresis in Tris-HCl buffer. Alkaline phosphatase has been stained by a modification of a histochemical method (Pearse, 1960) and lactic dehydrogenase by means of a tetrazolium salt in the presence of methylene blue. Vertical starch-gel electrophoresis has been employed (Smithies, 1959). It has been shown that extracts of human costochondral junction, liver and small intestinal mucosa show alkaline phosphatase activities which behave differently as a result of starch-gel electrophoresis. Intestinal mucosa gives two bands, one of which seems to be identical with that given by liver. The enzyme usually present in normal serum behaves in the same way as that in the liver extract, and only occasionally are activities demonstrable which correspond to those of small intestine and costochondral junction. It would thus appear that the enzyme present in normal human serum is derived mainly from the liver.

With regard to lactic dehydrogenase normal serum usually shows three or four bands on the anode side of the line of insertion. Heart extract shows four bands of activity; the most intense band is that nearest to the anode. A similar picture is obtained in the sera of patients who have had a recent myocardial infarction. Liver extract, in addition, shows a band of activity on the cathode side of the line of insertion, and the same band appears in the serum of patients suffering from liver disease.

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## An optical and electron microscope study of tooth structure

By E. H. Boult and C. H. Tonge. *Department of Chemistry and Department of Anatomy, King's College, University of Durham, Newcastle upon Tyne* 1

Recently extracted human teeth of known history have been bisected and ground sections have been prepared from one half. Silver shadowed optical and gold palladium pre-shadowed carbon electron microscope

replicas have been made from the polished surface of the other half. Micro-dissection of the tooth surface has been effected by progressive etching with varying dilutions of hydrochloric acid. Serial optical and electron microscope replicas have been made after each etch. The ground sections and optical replicas have been examined by light microscopy in order to select areas for detailed study, using the electron microscope. Thus the position and arrangement of the individual structures of the tooth were ascertained optically prior to the examination of similar fields with the electron microscope.

Tooth structure has been demonstrated and the arrangement of the enamel prisms in relationship to Schreger's lines demonstrated. Collagen has been shown in the matrix of cementum and dentine, and on comparison with collagen from the tendo-calcaneus shows the same characteristics.

**A portable apparatus for recording the rate of clearance of radioactive sodium from human calf muscle**

By L. Molyneux, J. Turnbull and D. N. Walder. *Departments of Physics and Surgery, The Medical School, University of Durham*

**A linear to logarithmic convertor unit for use with a linear counting rate-meter**

By J. Turnbull and D. N. Walder. *Department of Surgery, The Medical School, University of Durham*

**Studies of the salt glands of birds**

By R. J. Scothorne. *Department of Anatomy, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**The distribution of cholinesterase in the central nervous system**

By R. S. Snell. *Department of Anatomy, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**The blood supply of normal bone and bone affected by Paget's disease**

By T. E. Barlow and C. C. Slack. *Department of Anatomy, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**The blood supply of the stomach and colon in man**

By T. E. Barlow. *Department of Anatomy, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**A simple method of measuring respiratory volumes**

By E. A. Cooper and E. A. Pask. *Department of Anaesthetics, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**A method of collecting separately parotid and mandibular saliva and of measuring its pH without contact with air**

By C. Dawes. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**Class apparatus. A simple arrangement for measuring membrane potentials; a low-rate Geiger counter recording on a kymograph; a four-element thermistor bridge for skin temperature measurement**

By W. T. Catton. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**Single unit responses from frog skin mechanoreceptors**

By W. T. Catton. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne,* 1

**An effect of taurocholate on amino-acid production by small intestine *in vitro***

By A. M. Dawson and D. M. Matthews. *Department of Medicine and Department of Patho ogy, The Royal Free Hospital, London, W.C.* 1

When everted sacs or strips of rat small intestine are incubated in Krebs–Ringer phosphate saline without added substrate, appreciable quantities of free amino acids appear in the fluids bathing the preparation. The amino acids appearing in highest concentration are alanine, leucine, valine, glycine, glutamic acid and histidine. In the case of sacs, the amino acids are more concentrated on the serosal side.

The addition of sodium taurocholate in physiological concentrations of 5 mM and above causes a large increase in the concentration of all the amino acids appearing in the fluids bathing the gut. This can be shown by paper chromatography and followed by the colorimetric ninhydrin reaction. The effect is illustrated in Table 1. This effect might be due to a direct action of taurocholate in accelerating proteolysis (Noll, 1953) in the fluids bathing the gut or within the intestinal cells, but it has been found that addition of taurocholate to cell-free homogenates of intestinal mucosa has no effect in accelerating proteolysis. Alternatively, it might

be due to increased permeability of the intestinal cells, leading to 'leakage' of amino acids from the cells into the surrounding fluid. If this is so, the intracellular amino-acid concentration must be maintained by increased break-down of protein or peptides within the cells, since the amino-acid concentration in the intestinal wall does not fall.

TABLE 1. Concentration of amino acids appearing in fluids bathing sacs of rat small intestine, incubated with and without taurocholate

(The sacs were incubated in Krebs–Ringer phosphate saline for 1 hr at 32° in air. Initial mucosal volume: 3 ml.; initial serosal volume: 0·5 ml. Amino-acid concentrations were estimated by the ninhydrin reaction and are expressed as mg/ml. of leucine. Sac wt. ca. 300 mg.)

| | | Amino-acid concentration (mg/ml.) | | |
|---|---|---|---|---|
| | Sac no. | Mucosal fluid | Serosal fluid | Sac wall |
| Controls | 1 | 0·29 | 0·71 | 3·1 |
| | 2 | 0·29 | 0·84 | 2·8 |
| | 3 | 0·36 | 0·64 | 2·8 |
| | 4 | 0·25 | 0·57 | 3·5 |
| | | | Mean | 3·05 |
| Plus Na taurocholate 20 mM | 5 | 0·50 | 1·20 | 3·3 |
| | 6 | 0·55 | 0·80 | 3·0 |
| | 7 | 0·71 | 1·03 | 3·0 |
| | 8 | 0·65 | 1·10 | 3·0 |
| | | | Mean | 3·07 |

Tween 80 has no effect on amino-acid production by isolated gut. Sodium deoxycholate produces some increase in the amino acids appearing in the mucosal fluid, but in contrast to the conjugated bile salt damages the intestinal mucosa.

This work was supported by a grant from the Medical Research Council.

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## The influence of ascorbic acid on corticosteroid control of mitosis rates in regenerating liver

By J. T. HEMINGWAY and A. N. HOWARD. *The Department of Physiology, Queen's College, Dundee, and the Dunne Nutrition Laboratory, Cambridge*

In previous work (Hemingway, 1960), adrenalectomized rats subjected to partial hepatectomy showed a liver regeneration rate at least double that of rats with intact adrenals. Also rats implanted with growing tumours when partially hepatectomized showed a similar high regeneration rate. Corticosteroids seemed either deficient or inactivated in tumour-bearing rats.

Some rats given 200 r whole-body X irradiation showed these high mitosis rates, contrary to the usual inhibition (Hemingway, 1959). The possibility was suggested that oxidation processes in plasma interfere with normal states of reduction in which corticosteroids could exert their effect on cells. Ascorbic acid was a likely redox substance to be affected, so liver mitosis rates in scorbutic and normal guinea-pigs were compared after partial hepatectomy. It seemed that a 'functional adrenalectomy' may be caused by deprivation of ascorbic acid so that liver regeneration in a scorbutic guinea-pig might reveal a doubling of mitosis rate over that of a normal animal.

Owing to operative difficulties in scorbutic animals, very few of these survived, but three scorbutic animals showed mitosis rates averaging 52 whereas the normal average was 24 mitoses per 1000 cells.

To confirm this earlier work a larger experiment is now reported with 24 normal guinea-pigs having mean regeneration rates of 23 mitoses per 1000 cells. Of 48 scorbutic guinea-pigs 18 survived with mitosis rates averaging 51 per 1000 cells (S.D. $\pm 5$). A third group, not scorbutic, but partially starved to amounts of food eaten by scorbutic animals showed mild stress effects with mitosis rates averaging 15 per 1000 cells (S.D. $\pm 8$).

Lack of reduced ascorbic acid in plasma may prevent the inhibiting action of corticosteroids on mitoses in regenerating liver.

Support by the Medical Research Council is gratefully acknowledged.

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## The effects of penicillin on evoked cortical responses in the cat

By C. A. ESBERARD,* G. A. GERMAN† and W. LIFSCHITZ‡. *Department of Physiology, University of Aberdeen*

Walker & Johnson (1946) and Ralston (1958) showed that topical application of penicillin to the cerebral cortex produced muscular convulsions, with corresponding encephalographic activity, in man and animals. The experiments reported here were designed to ascertain the effect on the evoked cortical response, of this substance in concentrations insufficient to produce convulsive effects.

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Recordings from the somaesthetic area of the cerebral cortex of cats anaesthetized with chloralose or Nembutal (pentobarbitone; Abbott Laboratories) were made at the surface and at different levels below the cortical surface, using a micro-electrode filled with 3 M-KCl. The sodium salt of benzyl-penicillin, in concentrations of $2{\cdot}9 \times 10^{-3}$ to $2{\cdot}9 \times 10^{-2}$ M in C.S.F. Locke, was applied to the cortex beneath a pool of paraffin. The time of onset of the effects due to penicillin varied from animal to animal and depended on the anaesthetic used (0·5–4 min with chloralose, 2–10 min with Nembutal).

In animals under chloralose anaesthesia, the first and most consistent change to appear was a late diphasic wave, initially positive when recorded below the reversal point of the response evoked by contralateral forepaw stimulation, and initially negative when recorded above it. On first appearance of this wave the latency was variable but later it became constant. It varied from animal to animal so that sometimes it fused with the initial evoked response and on other occasions it was delayed by as much as 70 msec. Spike activity always occurred with the maximum negative deflexion of the penicillin-induced wave.

Increasing the frequency of stimulation from 32/min, which was normally employed, to 128/min caused a progressive increase in latency of the penicillin-induced component of the evoked response, while the remainder of the response stayed constant. As this penicillin-induced wave separated from the initial evoked response, a third wave, negative in polarity with a latency of 20 msec, appeared which, unlike the penicillin-induced wave, was abolished by intravenous D-tubocurarine.

The same general effect was observed under Nembutal anaesthesia but the penicillin-induced component appeared with a shorter latency, fusing almost completely with the initial evoked response. In this case, the first effect of penicillin was an increase in the negative component of the initial evoked response, in contrast to what was seen under chloralose anaesthesia, where the initial evoked response was practically unchanged. Under Nembutal anaesthesia increasing stimulation frequency produced no increase in latency of the penicillin-induced component.

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## The effect of gravity on gastric emptying

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## Studies on the mechanism of anaphylaxis in the rat

By C. F. Code, D. T. Cody and J. C. Kennedy. *Mayo Foundation, Rochester, Minnesota, U.S.A.*

A series of experiments was undertaken to determine whether the supersensitivity which follows the administration of *Bordetella* (*haemophilus*) *pertussis* vaccine (HPV) in rats is produced through the same mechanism as the supersensitivity that follows adrenalectomy and to learn something of the importance of histamine in the development of the symptoms of anaphylaxis in rats.

In adrenalectomized rats which have been sensitized by repeated injections of horse serum, cortisone given just before challenge reduces the incidence of death but does not prevent the occurrence of severe acute anaphylactic reactions, whereas cortisone given at the time of the sensitizing injections virtually eliminates acute reactions (Dews & Code, 1953). In contrast, intact rats sensitized to horse serum with the aid of HPV were protected against anaphylaxis by cortisone given just before challenge, but not when the cortisone was given with the sensitizing injections.

Administration of adequate quantities of adrenal cortical extract to adrenalectomized rats restores their resistance to injections of histamine (Marmorston-Gottesman & Perla, 1931). In contrast, cortisone given just before challenge by histamine to rats rendered susceptible by injections of HPV had only a very slight protective effect against histamine toxicity; and when cortisone was administered at the time of the vaccine, susceptibility to histamine toxicity was greatly increased.

During anaphylactic shock in rats treated with HPV, the increased concentrations of histamine and histaminase in the blood were not altered by the administration of cortisone just before challenge, although the symptoms of anaphylaxis were reduced.

When aminoguanidine was injected just before challenge, no histaminase activity was found in the blood during anaphylactic shock and the concentration of histamine was greater than that encountered in similar rats not given aminoguanidine. Despite this effect on the blood histamine, the aminoguanidine had no effect on the severity of the anaphylactic reaction.

It is concluded that HPV does not lower the resistance of the rat to sensitization and to histamine through the adrenal mechanism and that histamine does not seem to have a major role in the production of the symptoms of anaphylaxis in the rat.

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## The actions of certain amino acids on cortical neurones

By K. KRNJEVIĆ and J. W. PHILLIS*. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

The chemical sensitivity of single cortical neurones can be studied by applying various substances electrophoretically with multi-barrelled micropipettes inserted into the brain (Krnjević & Phillis, 1961). We have thus found that cells in the cerebral and cerebellar cortex of the cat and rabbit are readily excited by the dicarboxylic acids glutamic and aspartic, and the sulphonated analogue cysteic acid. On the other hand, the same cells are strongly inhibited by $\gamma$-amino-butyric acid and $\beta$-alanine, which are produced by $\alpha$-decarboxylation of the excitatory dicarboxylic acids. In this respect, cortical neurones resemble spinal neurones (Curtis & Watkins, 1960). However, cortical neurones differ in being relatively insensitive to any inhibitory action of taurine and $\gamma$-aminopropane sulphonic acid, the products of $\alpha$-decarboxylation of the sulphonated excitatory amino acids cysteic and homocysteic.

Cortical cells are unlike spinal neurones in several other respects. For instance, most cells are excited by asparagine and inhibited by glycocyamine, the guanadino derivative of glycine (cf. Purpura, Girado, Smith, Callan & Grundfest, 1959). Furthermore, whereas spinal neurones are strongly excited by the L and D enantiomorphs of glutamate and aspartate (Curtis & Watkins, 1960) cortical cells are very much more sensitive to the L than to the D form of glutamate, the usual sequence in descending order of activity being

L-glutamate > L-aspartate > D-aspartate $\gg$ D-glutamate.

The readiness with which cortical cells are excited by L-glutamate is clearly strong evidence in support of the suggestion made by Van Harreveld (1959) that glutamate is a factor in the causation of spreading depression; cortical neurones can readily be blocked by the passage of an excess of glutamate. In contrast, K ions applied electrophoretically in comparable amounts are much less effective in exciting cells. Our results are consistent with Van Harreveld's explanation for the greater effectiveness of D-glutamate in producing spreading depression when applied topically, that L-glutamate is probably metabolized rapidly by L-specific enzymes, which thus reduce its concentration around the cells.

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## Further purification of an active lipid acid from rabbit brain

By N. AMBACHE and MARGARET REYNOLDS. *Medical Research Council Department of Physiology, Royal College of Surgeons of England, London, W.C. 2*

Aqueous extracts of Locke-perfused rabbit brain contain a spasmogenic unsaturated hydroxy-acid (Ambache & Reynolds, 1960). Extracts were partitioned at pH 7·5 with 1 vol. ether freed from peroxides by passage through Woelm neutral alumina and redistillation. The inactive ether phase was discarded and partition repeated at pH 3 with another 1 vol. ether. The active residue obtained by evaporating this ether in nitrogen was dried *in vacuo* and re-extracted with a little ether. This solution (E)

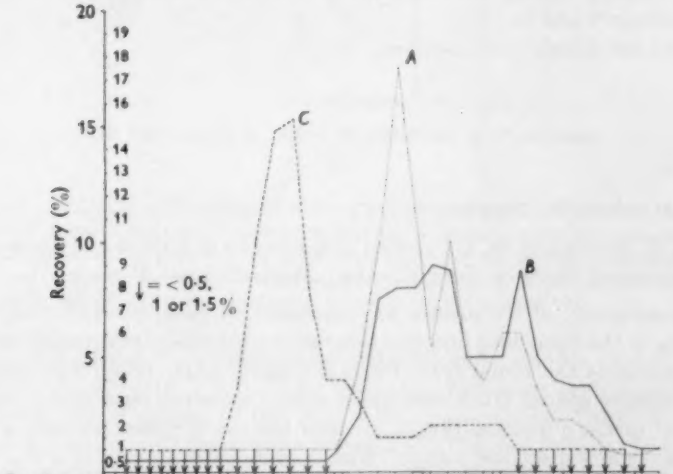


Fig. 1

was centrifuged clear and applied to columns of Hopkin Williams chromatography grade silica suspended in ether. The columns (diam. 5 mm; height, 8–10 cm) were eluted successively first with 100% ether, then with 5, 10, 15, 20, 25 and 30%, respectively, of redistilled methanol in ether. In four of five experiments the activity, assayed on atropinized hamster colons, appeared in the 10% methanol and subsequent fractions: in the fifth, in 15% methanol *et seq.* (Fig. 1*A*). When the ethereal solution E was not centrifuged before chromatography an active component appeared in fractions 1 and 2. This seems to consist of a material which had been soluble in the wet acid ether during the partitions, but later insoluble in anhydrous ether and removable as a greyish precipitate.

In four comparable experiments irin purified by partition into ether at pH 3 appeared in the 15% methanol and subsequent fractions (Fig. 1*B*); occasionally a trace appeared in the first 5% methanol fraction. Aqueous brain extracts were also purified by treatment with 20 vol. acetone and evaporation of the centrifuged acetone to a residue. In two chromatographic experiments with this type of material activity first appeared at 10% methanol (Fig. 1*C*) and in a third, at 15%.

Thus the active material from brain appears to have a polarity of the same order or slightly less than that of irin, but > ricinoleic acid (mostly in fractions 2 and 7).

Brain sulphatide was inactive.

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## Visual acuity in insects

By E. T. Burtt and W. T. Catton. *Departments of Zoology and Physiology, University of Durham, King's College, Newcastle upon Tyne*, 1

Visual acuity of the insects was measured by using the spike impulses arising in the optic lobe and thoracic nerve cord when illuminated objects are moved in the visual field (Burtt & Catton, 1954, 1959). The stimulus was equally spaced black and white stripes mounted on a sliding carrier moved across a window (Fig. 1A); thus the eye is presented only with a set of rapidly displaced stripes. Nine locusts gave a mean of $0{\cdot}3° \pm 0{\cdot}08°$ for the smallest angle ($\theta$) between adjacent black stripes giving five or more responses in ten trials. Two species of *Diptera* gave 0·28° and 0·3°. These values are much lower than those deduced from behaviour experiments (e.g. 1° in the bee, Hecht & Wolf, 1929) which have been related to the angular separation of adjacent ommatidia. In the locust the latter value is 1°–2·4°, and comparable in the two *Diptera*.

Eye slices were examined under the microscope to find the images produced by a striped object held in the understage carrier (Fig. 1B). Each lenslet forms a separate image (Fig. 1B, 1) but this only allows resolution of stripes with an angular separation of more than 1°; at less

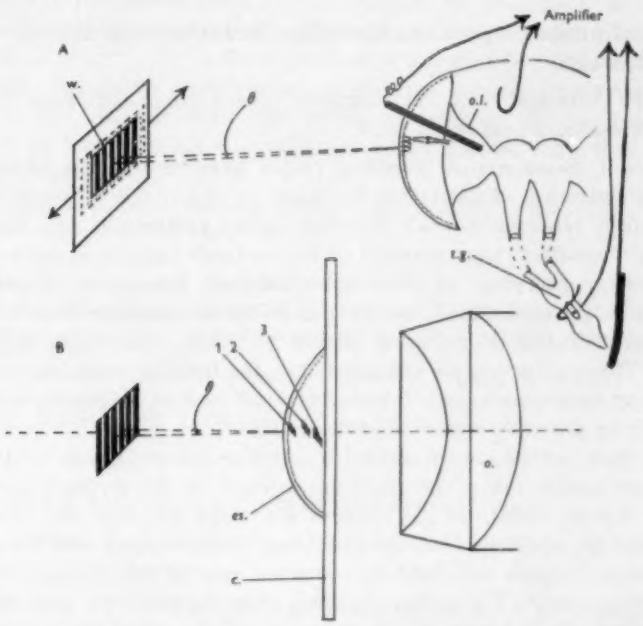


Fig. 1. (A) Equally spaced black and white stripes subtending an angle ($\theta$) at the eye, moved across a window (*w*) used as a stimulus to produce spike responses in either optic lobe (*o.l.*) or thoracic nerve cord (*t.g.*). (B) Similar stripes forming images (1, 2, 3) in an eye slice (*es.*) arranged as a hanging-drop preparation on the coverslip (*c.*) viewed by the objective (*o.*). Size of images exaggerated.

than 1° the image of the stripes becomes blurred. Deeper images are formed in the eye (Fig. 1B, 2, 3), lie within the basement membrane and give resolution of stripes of 0·5° and 0·35°, respectively. These images are due to interference phenomena arising from the first images. They do not always closely resemble the objects in the visual field; but they do allow resolution of stripes and could account for the acuity observed in the living preparation.

We are indebted to Dr G. L. Rogers, College of Advanced Technology, Birmingham, for collaboration in optics and to the Anti-Locust Research Centre for apparatus.

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## Effect of pregnancy on the histidine decarboxylase activity of the rat's stomach

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Kahlson, Rosengren & Westling (1958) have shown that in rats the urinary excretion of histamine increases on about the fifteenth day of pregnancy, reaching a peak the day before parturition and declining rapidly thereafter; that removal of the foetuses leads to a reduction of the urinary excretion of histamine (Kahlson, Rosengren, Westling & White, 1958), and that foetal liver is richer in histidine decarboxylase than other foetal or maternal tissues (Kahlson, Rosengren & White, 1960). These observations indicated that the foetuses were the principal source of histamine excreted during the last part of pregnancy and that foetal liver probably contributed most of it.

The most potent known source of histidine decarboxylase in the non-pregnant female rat is the glandular portion of the stomach (Schayer, 1956; Waton, 1956). In the present study the histidine decarboxylase activities of stomachs from pregnant and non-pregnant rats have been compared. Virginal and bred female white rats of two Sprague–Dawley strains were used. Tissue was obtained after the rats were anaesthetized with ether. In the case of the stomach, all the glandular portion was used. In a few instances, tissue was frozen before use; more often it was homogenized while fresh. Histidine decarboxylase activity was measured by a modification of the method of Waton (1956).

The mean histidine decarboxylase activity of stomachs from eighteen female non-pregnant rats was 18·7 $\mu$g of histamine formed/g/hr (S.E. $\pm$ 1·95); the comparable value for thirteen rats between the seventeenth and twenty-first days of pregnancy was 71·0 (S.E. $\pm$ 12·36). The mean difference of 52·3 is significant ($P < 0{\cdot}001$). More histamine could be extracted from stomachs of pregnant rats (80·5 $\mu$g/g $\pm$ 8·27) than non-pregnant female rats (30·4 $\mu$g/g $\pm$ 2·85), and again the difference between the means (50·1) is significant ($P < 0{\cdot}001$). Foetal livers yielded the enzyme in higher quantities than did maternal stomachs, the mean value for the livers of thirteen litters being 139·3 $\mu$g/g/hr (S.E. $\pm$ 14·13), but their mean content of histamine was lower (30·4 $\pm$ 2·85). Tests on four animals indicated that no histidine decarboxylase was detected in foetal

placenta or maternal liver; activity in foetal carcases without liver averaged only 1·4 $\mu$g/g/hr.

These findings confirm the observations of Kahlson and associates that foetal liver is the richest source of histidine decarboxylase during the last third of pregnancy in the rat and add that at the same time a nearly fourfold increase in the activity of the enzyme also occurs in maternal stomach. The data do not define the role of the increase in enzyme in the stomach. It may be more concerned with the delivery of histamine for local storage and use than that of the foetal liver, for the histamine content of the maternal stomachs was much greater than that of the foetal livers.

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## Blood–brain barrier and extracellular space

By H. Davson, C. R. Kleeman and E. Levin. *Department of Physiology, University College London*

It has been argued that the blood–brain barrier is not an expression of an impedance to diffusion across the cerebral capillaries but is due to an absence of a significant extracellular space in the tissue; thus the failure of sucrose and *p*-aminohippurate (PAH) to penetrate from blood to the nervous tissue to an appreciable extent is said to be due to their being 'extracellular' substances and thus excluded from the water of the nervous tissue, which is said to be almost entirely intracellular. Evidence against this argument was presented by Davson & Spaziani (1959) who showed that these extracellular substances passed rapidly into pieces of cerebral hemisphere, maintained in Krebs–Ringer's solution to which the substances had been added. It can be argued, however, that the tissue under these conditions was abnormal, so that the experiment has now been repeated by an *in vivo* technique, the cerebrospinal fluid of the anaesthetized rabbit being repeatedly replaced with a Ringer's solution containing PAH and sucrose. After 2–3 hr the brain and spinal cord were removed and the concentrations of PAH and sucrose in the tissues and the Ringer's solution compared. From these results PAH and sucrose spaces

were computed for cord and brain; these are shown in Table 1. The cord has spaces in the region of 12%. The low figures for the brain are due to the circumstance that the replacement fluid injected into the cisterna magna does not reach the convexities of the cerebellum and cerebral

TABLE 1. PAH- and sucrose-spaces of brain and cord

| Expt. no. | Substance | Space (%) Brain | Space (%) Cord | Duration of expt. (hr) |
|---|---|---|---|---|
| 1 | Sucrose | 2·2 | 14·1 | 2 |
| | PAH | 1·4 | 8·5 | |
| 2 | Sucrose | 4·9 | 12·3 | 2 |
| | PAH | 3·5 | 11·6 | |
| 3 | Sucrose | 6·6 | 10·3 | 2 |
| | PAH | 1·7 | 12·5 | |
| 4 | Sucrose | — | 12·0 | 3 |
| | PAH | — | 10·8 | |

cortex, as is shown by injected dyes in control experiments; by contrast the cord is well bathed throughout its length by the replacement-fluid. Since the blood–brain barrier extends to the cord, it is difficult to escape the conclusion that the failure of PAH, sucrose, etc., to penetrate the nervous tissue of brain and cord to an appreciable extent is due to some impediment to their passage from the blood, i.e. there *is* a blood–brain barrier.

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## An analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors

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In dogs, spontaneously breathing room air, stimulation of the carotid bodies by hypoxic blood causes cardiovascular effects due to at least two mechanisms occurring simultaneously: (1) a primary cardiovascular reflex arising from the chemoreceptors themselves, and (2) events arising secondarily from the concomitant reflex increase in respiratory minute volume (Daly & Scott, 1958, 1960). These secondary effects may be excluded by maintaining pulmonary ventilation constant by means of a pump.

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The primary cardiovascular reflex effects include bradycardia and peripheral vasoconstriction, and the present investigation was made to analyse the nervous pathways through which these responses are mediated.

Dogs were anaesthetized with either pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd., 45 mg/kg) or a mixture of chloralose (0·05 g/kg) and urethane (0·5 g/kg) intravenously, preceded by morphine hydrochloride (2 mg/kg) subcutaneously. They were artificially ventilated by means of a Starling 'Ideal' pump and were given decamethonium iodide (0·25 mg/kg) to prevent spontaneous respiratory efforts occurring during stimulation of the chemoreceptors. The carotid body chemoreceptors, isolated from the circulation, were stimulated by changing the perfusate from oxygenated to hypoxic blood obtained from a donor dog (Daly & Scott, 1958). The carotid sinus pressure was automatically maintained at the same level as the systemic blood pressure. The vascular resistance in one limb was measured by isolating the limb from the circulation and perfusing it by means of a Dale–Schuster pump at constant blood volume inflow from a second donor dog. In other experiments, the blood flow in a femoral or inferior mesenteric artery was continuously recorded by means of a rotameter.

Stimulation of the carotid bodies invariably caused bradycardia, and an increase in vascular resistance in the limb and splanchnic area.

The peripheral vasoconstriction was abolished by the administration of hexamethonium or by denervation of the vascular territory being perfused, indicating that it is mediated via the sympathetic nervous system, as found previously by Bernthal (1938) and by Bernthal & Schwind (1945). On the other hand, the bradycardia resulting from chemoreceptor stimulation was often reduced but not abolished by division of the cervical vagosympathetic nerves. This residual response could be abolished either by hexamethonium or by applying a local anaesthetic to the stellate ganglia.

These results indicate that the slowing of the heart is due both to an increase in vagal tone and to a reduction in sympathetic tone. It is of interest, however, that whereas stimulation of the carotid bodies reflexly reduces the cardiac sympathetic activity, the activity in sympathetic nerves to blood vessels in the limb and splanchnic area is simultaneously increased.

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## Muscle blood flow during exercise in patients with intermittent claudication

By D. N. WALDER. *Department of Surgery, The Medical School, Newcastle upon Tyne*

Techniques of estimating calf muscle blood flow, which depend on measuring the changes in volume of the limb during venous occlusion, are difficult to apply when the patient is exercising, especially if the exercise consists of walking.

It has been established that, with certain provisos, muscle blood flow is related to the clearance rate of a depot of radioactive sodium chloride previously injected into a muscle (Walder, 1955). A portable apparatus, which can be carried in a pack on the subject's back, has been developed for recording the rate of clearance of radioactive sodium from the calf muscle (Molyneux, Turnbull & Walder, 1958), thus enabling the pattern of blood flow before, during and after various types of exercise in normal subjects and patients with intermittent claudication to be studied.

In a patient who claudicates, it can be shown that at rest there is little impairment of blood flow compared with a normal subject. During exercise, however, impairment of the blood flow is at once obvious and, in some circumstances, blood flow actually ceases. At the end of exercise, a period of rapid flow comparable to that of a normal subject occurs.

An attempt has been made to explain this markedly diminished flow during exercise in patients with claudication. One factor thought to influence muscle blood flow is the intramuscular tension developed during contraction (Barcroft & Millen, 1939). Experiments in which normal subjects and patients with intermittent claudication made a series of increasingly strong sustained contractions of the calf muscle showed that for any given contraction the muscle blood flow was less in those with claudication than in normal subjects, and that muscle blood flow ceased at a strength of contraction which was less in claudicants than in normal subjects. When the strength of contraction is such that no blood flow occurs, the intramuscular tension is presumably greater than the pressure in the nutrient vessel.

It is therefore concluded that in patients who claudicate a subnormal pressure in the nutrient artery is the determining factor. At rest and immediately after exercise, when intramuscular tension is low, the effect of a lowered pressure in the nutrient artery is minimal and flow under these conditions is little affected.

This conception of the cause of claudication in exercise allows a logical explanation for the unsatisfactory results to be obtained from the use of muscle vasodilator drugs and the operation of lumbar sympathectomy in

this condition and also for the fact that a patient can claudicate in spite of an apparently anatomically normal muscle vasculature.

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## The histamine content of gastric juice during gastrin stimulation

By BRAHMADUTT, A. P. DOUGLAS and H. J. LAKE. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

Important evidence in favour of the hypothesis (Babkin, 1938) that histamine is released as a local agent at the parietal cell is that gastric juice, however stimulated, contains histamine. Code (1956) observed, however, that the juice secreted by dogs in response to a meat meal had a ratio of histamine-to-acid output which was greatest in the first ½ hr after feeding, much less in the second ½ hr and least in subsequent periods. This suggests that histamine may not be as important in the chemical as in the nervous phase of gastric secretion. Support for this interpretation came from Kaulbersz & Bugajski (1956) who found that in dogs gastric secretion contained 34 ng of histamine/ml. after vagal stimulation and only 1·2 ng/ml. in response to gastrin. Emmelin & Kahlson (1944), however, found in anaesthetized cat a wide range of histamine concentrations in both vagal (4–150 ng/ml.) and gastrin (20–55 ng/ml.) stimulated secretion. This matter has been investigated further with a histamine-free gastrin which does not depend for its action upon a generalized release of histamine (Blair, Brahmadutt, Harper & Lake, 1961).

Experiments were performed on anaesthetized cats with their vagus and splanchnic nerves cut. The stomach was intubated via the oesophagus and the pylorus occluded. An isosmolar solution of hydrochloric acid in saline (pH 2·3) was run into the stomach and replaced at intervals. Samples of the washings were estimated for hydrogen ion and free histamine (Adam, Hardwick & Spencer, 1957). Gastrin was administered in saline intravenously at the rate of 3–5 mg in 45 min. Gastric washings were obtained both during gastrin stimulation and during control periods in which there was no stimulation and little or no spontaneous hydrogen ion secretion.

During the control periods the output of histamine was 15·3 ng (S.E. ± 2·6)/30 min and during active hydrogen ion secretion in response to gastrin stimulation it was 17·0 ng (S.E. ± 2·9)/30 min. There is no significant

difference between the means of the histamine content in these two groups. No correlation was found between histamine output and the amount of acid secreted in response to gastrin stimulation over the range 0·13–1·91 m-equiv HCl/30 min.

The low histamine content of gastrin-stimulated gastric secretion is in accordance with the findings of Kaulbersz & Bugajski, and suggests that the histamine content of gastric juice cannot be used as evidence for the view that histamine is an intermediary in the action of gastrin. These results also provide evidence that rates of acid secretion up to 1·91 m-equiv HCl/30 min do not lead to the release of histamine as a side effect of parietal cell activity (Gregory, 1955).

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## The effect of atropine upon gastrin-stimulated acid gastric secretion

By E. L. Blair, A. A. Harper, H. J. Lake and J. D. Reed. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

The gastrin of earlier workers may not have been inhibited by atropine because the extracts contained histamine. This observation has however been made in anaesthetized cats by Komarov (1942) using histamine-free extracts, and by Blair, Harper, Lake, Reed & Scratcherd (1961) using histamine-free extracts which do not depend for their action upon a generalized release of histamine (Blair, Brahmadutt, Harper & Lake, 1961). Nevertheless, Gregory & Tracy (1961) found that their histamine-free extracts were strongly inhibited by atropine in man and in the conscious dog. It seemed desirable to investigate further the effect of atropine upon gastrin stimulation in the anaesthetized cat.

Cats were anaesthetized with chloralose. The vagus and splanchnic nerves were cut in the majority of experiments, although these procedures did not appear to influence the results. The stomach was intubated via the oesophagus and the pylorus occluded. In a few experiments the external secretion of the pancreas was studied in addition to the gastric secretion. Gastrin was administered subcutaneously or intravenously.

Atropine sulphate up to 1 mg/kg body weight, intravenously, did not produce any significant effect upon intermittent or continuous gastrin-stimulated acid secretion in twenty cats. The range of acid outputs varied from 0·02 to 1·2 m-equiv/15 min. Maximal gastrin secretory responses were not determined, but in seven experiments with acid outputs less than 0·35 m-equiv/15 min, it was demonstrated that the secretory rate could be at least doubled by injecting larger doses of gastrin. Comparable rates of cholinergically stimulated acid secretion could be completely inhibited by atropine.

The discrepancy between these results and those of Gregory & Tracy does not depend on the method of preparing gastrin, since similar results were obtained with extracts made by the technique of Gregory & Tracy (1959). Like our extracts, these also stimulate pepsin secretion, stomach tone, the flow of pancreatic juice, and the output of amylase from the pancreas. All these effects can be obtained in the atropinized animal.

Muscarinic cholinergic excitation clearly does not play an essential part in the response of the parietal cell to gastrin in the anaesthetized cat. In conscious animals and man the inhibitory effect of atropine may not be due to direct interference with the action of gastrin on the parietal cell. It might possibly be secondary to the stimulant action of atropine on the vasomotor centre, resulting in constriction of the splanchnic vessels. Alternatively it might result from the blocking of a tonic sub-threshold cholinergically-mediated release of gastrin from the antrum by which the response to injected gastrin in man and conscious animals is enhanced.

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## The effect of type of stimulus on the composition of saliva

By C. Dawes and G. N. Jenkins. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

Pavlov (1910) stated that the composition of saliva in dogs was adapted to the nature of the stimulus, and Pickerill (1912) interpreted teleologically the high alkalinity of the saliva produced in human subjects in response to acid stimuli. The possibility that these reported differences in composition are related to rates of flow, rather than to specific stimuli, has not been

adequately investigated. Changes in the proportion of saliva derived from the parotid and submandibular glands with different stimuli would also modify composition. In man there have been few studies in which the response of the different glands to a variety of stimuli have been compared.

Saliva has been collected separately from the parotid and submandibular ducts after a variety of stimuli each at several levels of intensity. The concentrations of sodium, potassium and phosphate were found to be related to rates of flow irrespective of the nature of the stimulus, i.e. there was no evidence of specific adaptation to different stimuli. The only striking result with calcium was that the concentration in submandibular saliva was three times greater than in parotid saliva.

The concentration of amylase in parotid saliva, however, was higher, at any particular rate of flow, when 'Oxo' was the stimulus than with fruit drops. Contrary to the findings of Ferguson, Krahn & Hildes (1958), the concentration of amylase was observed to rise with increasing rates of flow.

The pH value of 'resting' saliva, measured without contact with air, varied between 5·55 and 5·8 for parotid, and somewhat higher for submandibular saliva, rising to 7·9 with maximum rates of flow (confirming Schmidt-Nielsen, 1946). The pH was clearly related to the rate of flow and not to the pH of the stimulus. Even substances with pH values as widely separated as sodium bicarbonate and citric acid, in quantities which stimulated comparable rates of flow, caused the secretion of saliva with similar pH values.

In one subject with whom it has been possible to collect simultaneously from both parotid ducts and the joint submandibular ducts, different stimuli altered the proportional contribution from the two types of gland. In general, mainly mechanical stimuli (presence of sand, or chewing wax or bread) promote twice as much parotid as submandibular saliva, whereas strongly sapid materials stimulate both glands about equally. The composition of mixed saliva could, therefore, vary under the influence of different foods by this unequal response by the two types of gland.

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## The lability of plasma 'Porter Silber chromogens' in the peripheral circulation of normal subjects and psychiatric patients

By BERYL GLOCKLING, M. HARPER and M. ROTH. *Department of Psychological Medicine, Medical School, King's College, University of Durham, Newcastle upon Tyne*, 1

A relationship has been demonstrated between emotion and adrenocortical function by measuring steroid levels in urine and plasma (Mason, 1959; Persky, Korchin, Basowitz, Board, Sabshin, Hamburg & Grinker, 1959). The diurnal variation of plasma corticosteroid is also well established (Jenkins, 1960).

In the present investigation 'Porter Silber chromogens', believed to be principally hydrocortisone, have been measured by the method of Peterson (Peterson, Karrer & Guerra, 1957) in blood samples taken from the antecubital vein.

Many of the patients investigated have been suffering from a clearly defined neurotic illness, the phobic anxiety depersonalization syndrome, which is characterized by a lowered anxiety threshold to a wide variety of stimuli and situations. Samples taken at weekly intervals during treatment showed a relationship between a reduction in the amount of 'Porter Silber chromogens' and a satisfactory therapeutic response. Further studies on samples taken immediately before and after an intravenous injection of thiopentone, and also before and after electro-convulsive therapy modified with thiopentone and suxamethonium chloride showed that significant changes in plasma levels could occur over shorter intervals than had been expected. Injection of thiopentone generally produces a reduction, and an electrically induced convulsion usually an increase in the plasma level (Harper, Glocking & Roth, unpublished).

These unexpected variations in 'Porter Silber chromogen' levels over short periods were further investigated by making serial estimations on blood samples taken at approximately 10 min intervals for 1 hr in 8 normal subjects and 13 patients. The experiments were conducted between 9.30 a.m. and 10.30 a.m. A rating of the subjects' emotional state was carried out by the psychiatrist at intervals throughout the experiment and it was found that patients reacted in an individual and variable way to the stress of repeated venepuncture and some spontaneously ventilated emotionally distressing topics when engaged in general conversation.

Variations were observed in the plasma levels of both normal subjects and patients. In the patients not only was there a significantly greater liability of level, but the average level was also higher than in normal individuals.

These results support the suggestion (Prunty, 1960) that the estimation

of steroid levels under basal conditions may give misleading results. It is also apparent that the fluctuation in 'Porter Silber chromogens' over relatively short time intervals makes the interpretation of individual samples, particularly from anxious subjects, very difficult, even if venepuncture is always performed at the same time of the day.

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## The urinary excretion of human chorionic gonadotrophin in normal pregnancy

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The normal excretion levels of human chorionic gonadotrophin (HCG) have been determined by Loraine (1958) on a group of fourteen subjects. In five of these 24 hr urine collections were assayed at weekly intervals, and in a further nine subjects isolated determinations were made at various stages of pregnancy. Four of the five subjects studied throughout pregnancy were multiparous and only one primigravid. The majority of the nine subjects in whom isolated readings were made were multiparous. The average age was 30 years.

Recently six normally pregnant women in their first pregnancy were studied in Newcastle upon Tyne. Their ages ranged from 20 to 32 years. Twenty-four-hour specimens of urine were obtained at weekly intervals up to the 20th week of the pregnancy. The total volume of the sample was measured, and its H.C.G. content assayed by the method of Loraine (1950).

Table 1 shows the excretion levels in the Edinburgh and Newcastle upon Tyne groups for three separate stages of pregnancy. The mean excretion in the Newcastle upon Tyne normal subjects was significantly higher than that from the normal subjects in Edinburgh at all stages of pregnancy.

($P < 0{\cdot}001$ for the periods of gestation 7–20 weeks, 7–10 weeks and 11–15 weeks; $P < 0{\cdot}01$ for the period 16–20 weeks.)

This difference between centres is at first sight difficult to explain, but it is possible that factors such as age and parity of the subjects may have been responsible, in view of the fact that the series from Newcastle upon Tyne was a carefully selected group of young women in their first pregnancy, whereas the series from Edinburgh consisted mainly of older multiparous women. Investigations are at present in progress to determine the effect of age and parity on H.C.G. excretion in normal pregnancy.

TABLE 1. H.C.G. excretion in normally pregnant women according to stage of pregnancy

| Centre | Stage of pregnancy | No. of subjects | No. of readings | H.C.G. excretion, i.u./24 hr | |
|---|---|---|---|---|---|
| | | | | Mean ± S.E. of mean | Range |
| Newcastle | 7–10/52 | 4 | 9 | 53,400 ± 3,780 | 35,000–71,400 |
| Edinburgh | 7–10/52 | 6 | 14 | 29,190 ± 1,890 | 18,320–46,000 |
| Newcastle | 11–15/52 | 6 | 27 | 37,780 ± 1,360 | 11,520–82,810 |
| Edinburgh | 11–15/52 | 9 | 26 | 11,410 ± 1,400 | 4,500–32,740 |
| Newcastle | 16–20/52 | 6 | 25 | 9,120 ± 640 | 4,830–18,130 |
| Edinburgh | 16–20/52 | 9 | 19 | 6,760 ± 320 | 4,500–9,350 |

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## Electrolyte content and inulin space of the cat pancreas

By D. G. CLARK*, A. A. HARPER and T. SCRATCHERD. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

In connexion with an investigation of the electrical activity of the pancreas, the pancreatic intracellular electrolyte levels have been determined. Previous work has mostly been carried out on the whole gland (Oldfelt, 1943: Manery, 1954). The contribution of the extracellular fluid to the whole gland analysis of the cat pancreas has now been estimated by the inulin space technique.

In cats anaesthetized with chloralose the splanchnic nerves were cut and the renal vessels tied. Inulin solution (0·6 g/kg) was injected intravenously. After an equilibration period of 2·5–5 hr, the animals were killed by exsanguination, the pancreas removed, blotted gently, and as much fat and connective tissue as possible were dissected off. Sodium, potassium, chloride and inulin were estimated in both tissue and plasma, and the fat and water content of the pancreas measured (Table 1). As the

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fat content of the pancreas varies considerably, it is clear that the results are best expressed in terms of wet fat-free tissue (Hastings & Eichelberger, 1937).

In studies on the rat pancreas Schneyer & Schneyer (1960) have reported similar figures for potassium, chloride and inulin space, but an intracellular sodium concentration which is about half that in the cat.

TABLE 1. Composition of the cat pancreas (14 experiments)

| | Inulin space (ml./kg) | Fat (g/kg) | Water (ml./kg) | Intracellular composition (m-equiv/kg) | | |
|---|---|---|---|---|---|---|
| | | | | Na | K | Cl |
| Mean | 188·0 | 37·4 | 769·6 | 23·35 | 94·8 | 23·4 |
| S.D. | 34·2 | 27·90 | 13·5 | 7·85 | 7·40 | 4·16 |

Results expressed per kg wet fat-free tissue.

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## The effect of mesencephalic lesions or pinealectomy on the sodium retention produced by suprahepatic constriction of the inferior vena cava in rats

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It has been postulated (Farrell, 1960) that the pineal gland and/or the anterior central part of the mid-brain are necessary for the production of a trophic factor which controls the secretion of aldosterone. If this view is correct it may be expected that destruction of these areas will prevent the retention of sodium associated with the secondary hyperaldosteronism produced by suprahepatic constriction of the inferior vena cava (IVC).

To test this hypothesis the effect of suprahepatic constriction of the IVC has been studied in adult male albino rats which 1–2 weeks previously had been pinealectomized or given large electrolytic lesions stereotaxically placed in the anterior central part of the mid-brain. Urine samples were collected overnight (16 hr). The rats were deprived of food for 8 hr before starting the urine collection but water was given.

Sodium excretion ($U_{Na}V$) in rats which 5 days previously had been subjected to constriction of the IVC fell to a mean value of 33 $\mu$-equiv/16 hr from the control level of 509 $\mu$-equiv ($P < 0{\cdot}001$). The mean Na:K ratio fell from 0·59 to 0·035. An injection of the aldosterone-blocking agent, Spironolactone SC.8109 (5 mg/rat) caused a natriuresis in rats

with constriction of the IVC. ($U_{Na}V$ = 1412 $\mu$-equiv; Na:K = 2·15; $P < 0·001$) but had no significant effect in control rats ($U_{Na}V$ = 790; Na:K = 0·81; $P > 0·02$). Sodium excretion was found to be slightly higher in pinealectomized rats than in normal controls ($U_{Na}V$ = 912; Na:K = 0·93; $P < 0·01$), but when subjected to IVC constriction these animals developed severe sodium retention and ascites ($U_{Na}V$ = 49; Na:K = 0·039; $P < 0·001$). Similarly, sodium excretion in rats with mesencephalic lesions was greater than in control animals ($U_{Na}V$ = 1222; Na:K = 1·03; $P < 0·001$) but these rats also developed sodium retention and ascites when subjected to IVC constriction ($U_{Na}V$ = 128; Na:K = 0·078; $P < 0·001$). In both cases SC.8109 caused a great increase in sodium output with a fall in the excretion of potassium. In pinealectomized rats given SC.8109, $U_{Na}V$ = 1436; Na:K = 1·93; $P < 0·001$ and in rats with mid-brain lesions $U_{Na}V$ = 1896; Na:K = 1·97; $P < 0·001$.

In all animals with IVC constriction, venous pressure measured in the IVC by means of a saline manometer was found to be greatly increased. The mean value for twenty-three operated rats was 10·52 cm saline compared with 2·13 cm saline for ten normal animals ($P \ll 0·001$).

The results of these experiments suggest that the anterior central mid-brain area and the pineal gland cannot be the only sources of the adreno-glomerulotrophic hormone.

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## The influence of female sex hormones on melanin pigmentation of the skin

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Most of the observations on the influence of the female sex hormones on pigment formation have been macroscopic only and microscopical studies have been confined to vertical skin sections. Vertical sections have the disadvantage that accurate melanocyte counts are impossible and that the appearances of the melanocytes cannot be adequately demonstrated. The present research is a histochemical investigation into the appearances of the melanocytes in the skin of pure red and pure black female guinea-pigs, following ovariectomy and the administration of oestrogen or progesterone, using both skin sheets and vertical sections. Skin samples were removed from the ear, anterior abdominal wall, areola and sole of foot. Most of the samples were left intact, but a few were split,

by the technique of Billingham & Medawar (1951) as modified by Szabó (1955). All the samples were then processed under identical conditions using the dihydroxyphenylalanine reaction.

Following ovariectomy the melanin content of the melanocytes was reduced and many of the melanocytes became smaller in size and had shortened dendritic processes. Some melanocytes had a deformed appearance, which varied from a minor irregularity of the cell contour to an advanced degree of cell collapse with fragmentation of the dendritic processes. A significant reduction of the melanocyte count occurred only in the areola and this was accompanied by a reduction in the amount of free melanin.

In the ovariectomized animals which received oestrogen (oestradiol monobenzoate, 0·1 mg/day, intramuscularly 5 days a week for 4 weeks), the amount of melanin was increased both inside and outside the melanocytes in all the regions. The greatest increase occurred in the areola, where it was mainly due to a large increase of free melanin. No statistically significant changes in the counts were produced in any of the skin regions.

In the ovariectomized animals which received progesterone (0·4 mg/day, 5 days a week for 4 weeks) the general effect produced was similar but less than that which occurred after oestrogen treatment.

In the ovariectomized animals which received oestrogen and progesterone together in the same doses as when administered separately, the stimulating effect on melanogenesis was very much greater than when either hormone was given alone. The two hormones produced their greatest effect on the melanocytes in the skin of the areola. No statistically significant changes in the melanocyte counts were produced in any of the skin regions.

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## The influence of topical local anaesthetics on the inhibitory effect of low pH in the pyloric antrum on acid secretion

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The inhibitory effect of low pH in the pyloric antrum on gastrin-stimulated secretion of acid by the fundus may be consequent upon interference with gastrin release (Longhi, Greenlee, Bravo, Guerrero & Dragstedt, 1958) or the release of an inhibitory hormone to acid secretion (Harrison, Lakey & Hyde, 1956; Jordan & Sand, 1957). Nothing is known of the afferent side of this process.

The antral pH must be in the region of 1·5 before the inhibition of acid secretion is effective (Woodward, 1960). It seems impossible that this low pH could exist within the mucosa and it presumably acts on surface receptors. It is generally accepted that a local nervous pathway is concerned in the release of gastrin by food extractives, since their effect is prevented by topical application of local anaesthetics. The following experiments were carried out to see if a similar influence could be exerted on the inhibitory effect of acid.

Experiments were carried out on dogs. Irrigation of an antral pouch with 0·1 % ACh produced a plateau of acid secretion from a Heidenhain pouch. Acetylcholine is a gastrin-releasing agent which presumably acts close to or directly on the effector cell. Lim & Mozer (1950) briefly reported that its effect was not prevented by local cocaine. Its effect on acid secretion, however, is strongly inhibited by low antral pH (Kim, 1955).

Application of 5 % cocaine or 3 % lignocaine to the antral mucosa either before or for 1½ hr during the secretion in response to ACh did not affect the acid production, confirming the observation of Lim & Mozer (1950). Acidification of the ACh to pH 1·5 abolished the response within 1 hr. The anaesthetic agents applied for ½ hr before and during 1 hr of acidification did not affect the intensity or the rate of onset of the inhibition. Their ability to inhibit the effect on acid secretion of antral irrigation with meat extract was confirmed on the dogs used.

In the interpretation of these experiments it must be realized that the local anaesthetics are virtually inactive at low pH values. There is therefore no certainty of any effect on surface structures directly exposed to the acid irrigation. The local anaesthetics however have a high mucosal penetrant power and will become active as soon as they approach normal tissue pH values. The ability of both drugs to exert their effect when topically applied at low pH has been demonstrated by experiments in man in which they were applied to the gingival mucosa. Most subjects were unable to differentiate the anaesthetic effects of solutions of pH 1·5 and normal pH.

It is concluded that the experiments give no support to the possibility that an intramucosal nervous pathway is concerned in the inhibitory effect of acid in the antrum.

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## The electrolyte content of histamine-stimulated gastric secretion in the cat

By E. L. Blair and A. K. Yassin. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

In histamine-stimulated gastric juice, the rising hydrogen-ion concentration is linearly correlated with the falling sodium concentration (Hollander, 1961). This could be due to dilution of a sodium-containing non-parietal component by the parietal cell component, to a reduction in sodium output or to a combination of these. The present experiments reveal a reduced rate of sodium output during histamine-stimulated gastric secretion in the anaesthetized cat.

In thirty cats, anaesthetized with chloralose, the vagus and splanchnic nerves were cut and the bile and pancreatic ducts tied off. The stomach was intubated via the oesophagus, the upper end of which was ligated, and the pylorus was occluded. In six experiments the pyloric gland and parietal cell areas of the stomach were intubated separately and a ligature applied at their junction. Fifty millilitres of isosmotic glycine or mannitol solutions were run into the whole stomach or body of stomach and 5 or 10 ml. into the pyloric compartment. These solutions were replaced at 10 min intervals and samples taken for analysis. Histamine was given by slow intravenous injection in isotonic saline or glucose for periods up to 2 hr.

In contrast to the linear correlation which exists in these experiments between the outputs of potassium and of hydrogen ion during histamine stimulation (Blair, Harper, Harris, Reed & Wilkinson, 1960), there is no correlation between sodium and hydrogen-ion output at this time. Unrelated to the rate of hydrogen-ion output, the rate of sodium output falls to 45·6% (S.E. ±2·08) of that present under basal conditions. This lower output is reached within 10–20 min after starting the histamine injection and is maintained until a similar period after the infusion ceases. Both the pyloric gland area and the body of the stomach are important contributors to the sodium output during basal and stimulation periods. A similar percentage reduction in sodium output occurs in both compartments during stimulation.

As the absorption of sodium from the antrum and body of the stomach into the blood is greater during basal conditions than during acid secretion (Cope, Cohn & Brenizer, 1943), the reduced sodium output during histamine stimulation seems likely to be due to a reduced secretion rather than an increased reabsorption of sodium. As this reduction in sodium output also occurs in the pyloric antrum, it is not likely to be the result of an increased exchange of sodium in a postulated primary secretion of the 'gastron'

(Hirschowitz, 1961) for hydrogen ions from the parietal cells. The present evidence suggests that during histamine-stimulated gastric secretion there is a reduction in the contribution from a sodium-containing secretion common to the pyloric gland and parietal cell areas of the stomach.

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## Biliary and urinary metabolites of [4-$^{14}$C] corticosterone in the cat

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When [$^{14}$C]progesterone is injected intravenously, radioactive metabolites appear in bile. These account for the greater part of the progesterone in cats (Taylor & Scratcherd, 1960, 1961) and rodents (Shen, Elliott, Doisy & Doisy, 1954), and for about half the administered dose in man (Sandberg & Slaunwhite, 1958). However, up to 90% of other labelled steroids, including corticosteroids, is excreted in the urine of human subjects (see Sandberg & Slaunwhite, 1958, for refs.). The metabolism of [4-$^{14}$C]corticosterone in male cats has now been studied.

Animals were prepared as previously described (Taylor & Scratcherd, 1961), except that bile was collected by inserting a polythene tube in the common bile duct and tying off the cystic duct.

In two animals 0·11 mg (5 $\mu$c) [4-$^{14}$C]corticosterone in 0·2 ml. ethanol: 0·8 ml. saline was administered over 10 sec, and bile and urine were collected for 4 hr. Excretion of radioactivity was greater in bile (85·8, 90·9%) than in urine (1·1, 0·9%). Successive hourly samples of bile from one cat contained 59·3, 17·6, 6·1 and 2·8% of the dose respectively. Bile was collected from the second cat for three 20 min periods followed by three 1 hr periods; 0·9, 25·4, 21·3, 31·9, 8·0 and 3·4% of the dose appeared in these samples respectively.

In two animals 1·4 mg (5 $\mu$c) steroid in 0·5 ml. ethanol: 25 ml. saline was given as a continuous infusion over 45 min, and bile and urine were collected for 5 hr. Most of the radioactivity appeared in the bile (91·7, 88·8%) with little in the urine (0·6, 0·5%).

Neutral metabolites rendered soluble in ether: ethyl acetate (75:25) by successive hydrolytic procedures (Taylor & Scratcherd, 1961) were determined. Because of their low radioactivity content total urine samples

of each animal were combined for analysis, but bile samples collected over 0·5 or 1 hr were analysed. In bile from all animals unconjugated metabolites were absent: the proportion of glucuronides decreased with time after injection, water-soluble metabolites increased and cold-acid hydrolysed metabolites remained constant. In animals given a single dose of steroid, hot-acid hydrolysed metabolites decreased with time, whereas in the other pair of animals the proportion of these metabolites increased slightly in one cat and rose and fell in the other. In both bile and urine most of the radioactivity remained water-soluble after all forms of hydrolysis.

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## The anatomical basis of cutaneous sensation

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## The delayed polypnoeic response to heat in the shorn sheep

By J. Bligh. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

It has been shown that the onset of polypnoea in sheep can occur in the absence of any rise in the temperature of the blood supply to the brain, and it was assumed that the peripheral receptors involved must be located at some relatively naked area (Bligh, 1959). This has been confirmed by Waites (1961). If there are also warm-receptors beneath the fleece, its removal might enhance the onset of polypnoea. If, as Waites suggests, there are very few, the removal of the fleece might have no effect upon the response.

The removal of the fleece, or a large area of it, has been found to cause a reduction in respiratory frequency at room temperature (20° C) from a labile value of about 90/min to a very steady value of 12–20/min. When the ambient temperature is raised to 42° C, there is no change in respiratory frequency for up to 50 min. The onset of polypnoea then occurs abruptly and rapidly reaches the same value as in the unshorn animal at that

ambient temperature. This effect can be completely reversed by covering the shaved area with an insulating coat overnight and keeping it in position during the experiment. As the fleece regrows, the delay in the polypnoeic response to a high ambient temperature shortens and disappears. The respiratory frequency at 20° C then begins to rise. The normal pattern is restored when the fleece is about 2 cm thick. If the shaved area is progressively increased, a similar sequence is seen in reverse order. At first there is a progressive reduction in respiratory frequency at 20° C with no delay in the polypnoeic response to a raised ambient temperature. The delay then appears and lengthens as more fleece is removed. Although the removal of the fleece tends to cause a reduction in deep body temperature, measured in the brachiocephalic trunk, the change in respiratory pattern is apparently unrelated to this and shows a clear relationship only to the surface area involved, or to the uniform fleece length.

The exposure of cold receptors could possibly explain the reduction in respiratory frequency at 20° C, but the delayed response to heat and the abruptness of the onset of polypnoea at the end of the delay require some additional explanation.

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## Possible temperature-sensitive elements in or near the vena cava of sheep

By J. Bligh. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

When a high humidity condition is superimposed upon an already high ambient temperature there is a large secondary rise in respiratory frequency in the unanaesthetized sheep. A rise in deep body temperature also occurs when this last channel of heat loss is drastically reduced. In an attempt to assess its importance in bringing about this secondary rise in respiratory frequency, cold saline was infused into the vena cava at such a rate as to prevent any rise in the temperature of the blood supply to the brain during the first 20 min of the high humidity condition.

In some experiments the rise in respiratory frequency still occurred. Frequently, however, there was a sustained depression of respiratory frequency during the period of infusion with an immediate rise in respiratory frequency upon its cessation. The variability of this respiratory effect could not be related to the rate of infusion or to the position of the tip of the cannula in the vena cava. The infusion of an equal volume of saline at deep body temperature into the vena cava, and the perfusion of cold saline through a dummy cannula which passed through the skin to the

femoral vein and out again, were without effect upon the normal respiratory response to humidity.

It would seem that this depression of respiratory frequency is related only to the low temperature of the infusate, and indicates the presence of temperature-sensitive elements somewhere between the point of infusion in the vena cava and the left side of the heart.

A similar explanation has been offered by Blatteis (1960) for the shivering which resulted from the cold venous return from the hind limb of the dog but which could not be attributed to a change in brain temperature. Such temperature-sensitive elements are not necessarily specific temperature receptors concerned in the normal processes of thermoregulation, but their possible presence indicates the need for caution in interpreting the effects of warm- and cold-saline infusion and venous occlusion experiments in determining the relative roles of peripheral and hypothalamic temperature receptors.

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## The effect of heating the hypothalamus of the ox

By D. L. INGRAM and G. C. WHITTOW. *Department of Physiology, The Hannah Dairy Research Institute, Ayr*

It has been demonstrated that, although local heating of the hypothalamus of the ox is accompanied by panting, the increase in respiratory rate in response to an increase of environmental temperature occurs without any increase in the temperature of the blood (Bligh, 1957) or the hypothalamus (Findlay & Ingram, 1961). The present experiments were therefore designed to investigate further the part played by the heat sensitive region of the hypothalamus in the regulation of body temperature.

The hypothalamus was heated by means of an RF voltage across two implanted electrodes, and a thermocouple recorded the temperature to which the tissue was heated. In three animals, each aged 12 months, the hypothalamus was heated to 39·5°, 40·0°, 40·5°, 41·0° and 41·5° C, and measurements of respiratory rate, tidal volume and the temperature of some of the extremities were recorded. The experiments were performed at environmental temperatures of 0°, 15° and 30° C.

It was found that the effect of heating the hypothalamus depended both on the temperature to which it was heated and on the environmental temperature. When the ambient temperature was 0° C very little effect was noted on either respiratory rate or the skin temperatures of the ears and forelegs until the temperature of the hypothalamus was 41·5° C. But at 15° C, an increase in the temperature of the extremities, an increase

in respiratory rate and a decrease in tidal volume were noted at a hypothalamic temperature of 40·5° C. When the environmental temperature was 30° C the increase in respiratory rate during heating of the hypothalamus was noted at a hypothalamic temperature of 39·5° C. The increase was maximal at a hypothalamic temperature of 40·5°–41·0° C and tended to decrease at a temperature of 41·5° C. In one animal the tidal volume increased when the hypothalamus was heated while in the other two it decreased.

When the hypothalamus was heated during hyperthermia (body temperature above 40·0° C) respiratory rate was little affected, but sometimes decreased while tidal volume increased. A similar decrease in respiratory rate and an increase in tidal volume occurs also during severe hyperthermia in the ox when the temperature of the body reaches 40·5°–41·0° C (Findlay, 1957).

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## The topographical representation of the visual field on the lateral geniculate nucleus in the cat and monkey

By P. M. Daniel, D. I. B. Kerr, K. N. Seneviratne and D. Whitteridge. *Department of Neuropathology, Institute of Psychiatry, Maudsley Hospital, London, S.E. 5, and the Department of Physiology, University of Edinburgh*

We have mapped the projection of the visual field on the lateral geniculate nucleus in the cat and monkey, using a perimeter with small spots of light subtending 10′ to 1° 40′, and long stainless-steel micro-electrodes of about 7–10 MΩ impedance.

The salient feature of the lateral geniculate nucleus of the monkey is that all layers are quite independent of each other, and that the scale of the representation, i.e. the magnification factor (Daniel & Whitteridge, 1961), is the same for all the small-celled layers, i.e. VI, V, IV and III. In the large-celled layers, i.e. II and I, the scale is about the same for the peripheral field but the representation of the macula area is somewhat reduced.

We can confirm the findings of de Valois (1960) that cells which are excited only by narrow bands of colour are to be found in the small-celled layers. Layers VI and V contain predominantly 'on' centre units, those in IV and III are predominantly 'off' centre units. No narrow band receptors have been found in II and I, but 'on' and 'off' centre units are both common.

In the cat, layers A and $A_1$ contain cells excited from the contralateral and ipsilateral eye respectively. There is some tendency for 'on' and 'off' centre units to be grouped together. Layer B contains cells which give much more prolonged 'on' and particularly 'off' discharges in the dark. They have a threshold about 1 log. unit lower than the A units, larger receptive fields and less marked peripheral inhibition. With considerable background illumination they show much less activity (Hubel & Wiesel, 1961). Nothing similar has been seen in the monkey and it seems very likely that this layer is especially concerned in nocturnal vision.

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## Absence of early degeneration of fibres of the orbital part of the cat's optic nerve after transection of the intracranial part

By G. S. Brindley and D. I. Hamasaki. *School of Optometry, University of California, Berkeley, California, U.S.A.*

In the cat, degenerative changes (beading or fragmentation of fibres) can be demonstrated by the Nauta technique in the optic nerve or optic tract as early as the 4th day after enucleation of the eye, and by the 7th day the majority of the fibres of the nerve are very conspicuously degenerate. But if the nerve is cut through within the cranial cavity, no degeneration can be seen in Nauta preparations of its intraorbital part up to the 9th day. The first traces appear on the 10th day, and it is not until after the 12th that many fibres are beaded or fragmented.

We conclude either that there are no fibres in the cat's optic nerve whose cell bodies lie in the brain (contrary to the opinion of Cajal, 1894, and many later writers), or that such fibres exist but that *all* of them differ from the majority of those fibres whose cell bodies lie in the retina, and from other fibres of the cat's central nervous system, in that the Nauta technique fails to demonstrate any sign of degeneration until the 10th day after they have been separated from their cell bodies.

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## The production of beats by simultaneous stimulation of the human eye with intermittent light and alternating electric current

By G. S. Brindley. *Physiological Laboratory, University of Cambridge*

**A technique for the recording of pressure changes in the human alimentary tract, using a radiotelemetering capsule**

By M. RIDGWAY and A. N. SMITH. *Department of Medical Physics, University of Edinburgh and Gastrointestinal Unit, Western General Hospital, Edinburgh*

**Factors affecting emulsification of oleic acid**

By J. A. SAUNDERS. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne*, 1

**The action of acetaldehyde on the chemoreceptors of the carotid body**

By N. JOELS and E. NEIL. *Department of Physiology, Middlesex Hospital Medical School, London, W.* 1

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